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Some Enigmas Associated with the Genesis of Mammary Cancer in Mice*†

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(Received for publication June 24, 1948)

Any advances in our knowledge about the genesis of mammary cancer in mice may be attributed primarily to the availability of homozygous or inbred stocks. For research these strains are to the biologist as pure chemicals are to the chemist. If this analogy may be justified, one striking distinction should be mentioned, however. Whereas chemicals are generally stable over a long period of time, mice, like all living forms, are subject to "mutational" changes which may occur at any time regardless of the degree of inbreeding. Just as many investigators are interested in the biological effects of mutations induced by carcinogenic agents, investigators trained in genetics are constantly on the alert for mutations that occur spontaneously in control strains. If these are recognized they might compare with the synthesis of new chemicals, new "tools" for research; if they go unnoticed, the experimental results may be distorted because of the changes in the genetic constitution of the experimental animals and, therefore, may become the basis for new theories which cannot be confirmed.

One purpose of this report is to review the results that have been secured by various investigators on the development of mammary cancer in mice, another is to bring together in a single article the observations from many analogous experiments which are now scattered throughout the literature. Some additional material will be included.

In the biological approach to the study of the genesis of cancer, where the disease may result from the action and interaction of a number of causative factors, the number of variables may be controlled by the use of homozygous animals. Environmental factors which may influence the final results may be held constant by the observation of experimental and control animals during the same period in the same laboratory. Little (59) has stated that research with inbred stocks of mice "is a tool which enables us to hold genetics constant while we alter other factors such as hormonal level or other chemical or physiological variables. We can then hold these factors constant and by altering the genetic constitution obtain a vastly clearer picture of the relative role of not only each broad general field of agents but of the particular part played by individual components within these fields." Furthermore, Little (59) has cautioned those not acquainted with the principles of genetics about the development and continuation of inbred strains as follows: "It is well, however, to note that unless such strains are rigorously inbred by matings between sibs or between parents and offspring a very considerable degree of genetic heterogeneity may persist within any strain almost indefinitely. If close inbreeding is carefully carried out for fifteen or twenty generations without the introduction of any other type of mating, a very high degree of genetic uniformity will be obtained and can be maintained. This statement would, of course, have to be modified if one or more genes mutated. By this process the mutant genes would introduce once more a variation from the parental type. Continuation of the process of close inbreeding would, however, bring back homogeneity of either parental or the mutant type within a relatively short time."

Geneticists, Little, Strong, Haldane, Cloudman,

* Presidential address, American Association for Cancer Research, Inc., Atlantic City, March 12, 1948.

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and others (81, 63, 54–60, 47, 16) have acknowledged that the most sensitive physiological test now available to demonstrate the existence of sublines within a stock due to mutations, isolation of lines before the strain was homozygous or "pure," and/or uncontrolled matings, is obtained by the transplantation of tumors that developed spontaneously in the stock. In mice of the stock or line of origin, practically 100 per cent of the grafts will grow progressively; in unrelated stocks or genetically different sublines of the same strain, nearly all of the mice will be resistant. By this method (56) "One can now measure the difference between the strains of animals used and also, by using the same strain of animals, measure the change in the tumor itself. Thus one has a double check on the material with which one has worked." Also, "Within any such inbred strain the transplantation of tissue, either normal or tumorous, is ordinarily as successful as is the transplantation of a bit of similar tissue from one part of an individual's body to another. On the other hand, successful transplantation outside of the inbred strain is ordinarily much less frequent and is often entirely impossible" (57).

The genetic theory of transplantation, advanced by Strong and Little (81) and Little and Strong (63), as well as previous studies (3, 14, 16, 23), and the suggestions given above, have been the basis for further investigations to determine the homozygosity of various inbred strains of mice. Such differences have been noted and some experimental data will be cited to demonstrate that today sublines do exist within some of our inbred strains, as determined by the transplantation of tumors and perhaps as indicated by the incidence of spontaneous cancer. In considering the incidences in different studies it will be necessary to keep in mind that the animals might have been subjected to various environmental factors in different laboratories, for even in one inbred line the incidences may not remain constant over a long period (24).

Breeding females of the sublines of the Strong C3H stock (79) maintained by Andervont (3) and the writer (22) have been observed to have approximately the same incidence of mammary cancer and average cancer age; non-breeders of the Andervont line have a higher incidence and earlier average cancer age. Because Andervont found that spontaneous mammary tumors from mice of his line would not always grow progressively when transplanted into mice of our C3H line, he stated (3): "The colony is regarded as a separate line of C3H mice, and results obtained with it cannot be controlled by data from other lines of strain C3H mice." In 1944 (23) we reported on the transplantation of mammary tumors which had developed in mice of the C3H stock maintained by Strong and determined that they grew in either all or none of our C3H mice. Thus, the three lines must be considered as distinct sublines of the same stock although each should be regarded, until proven otherwise, as a homozygous line.

Adequate comparable tests have not been reported for sublines of the Strong A stock (79). The transplantation of either ovaries (49, 50) or mammary tumors would suggest that the mice of our cancerous and fostered lines of the A strain have the same genetic constitution, at least, as regards the tissues tested, although they have been separated for approximately 30 generations. Brain tumors induced in mice of this strain grew in all mice of our two sublines but similar results were not obtained when animals of the Kirschbaum line of the A stock were inoculated (Moore and others, unpublished data). Strong has reported that his A strain has a low incidence of mammary cancer at the present time (80).

Strong and Little (63, 81) showed that tumor dBrB would grow in all individuals of the dilute brown stock; ten years later (14, 15) the same tumor would grow in only about 80 per cent of the dilute brown mice of the Murray-Little line although all mice of the MacDowell line of the same strain were found to be susceptible (16).

During recent years several sublines have been isolated within the dilute brown stock by G. W. Woolley (85) and representatives of line 1 or 12 and 2 or 212 are being continued in Minnesota. In addition, we have mice descended from Murray's stock, obtained through the courtesy of S. G. Warner, which we have called line 8. Preliminary results on the transplantibility of tumors within two of these lines have been reported (23) and additional data are given in Table I. The tests showed that they should be considered as separate sublines since the 5 mammary tumors would not give progressive growths in all mice of the 3 sublines of the dilute brown stock.

Whether or not the genetic constitutions which determined the transplantibility of mammary tumors in the different sublines of the dilute brown stock might also influence the genetic susceptibility for spontaneous mammary cancer cannot be determined from the data given in Table II. However, there was no correlation between the incidences of mammary cancer in breeding and virgin females; for the mice of the line which showed the lowest incidence as non-breeders gave the highest incidence and lowest average age as breeders. Differences in the incidences by sublines have been suggested (85) and

TABLE I. RESULTS OBTAINED FOLLOWING THE INOCULATION OF MAMMARY TUMORS FROM MICE OF DIFFERENT SUBLINES OF THE D (DILUTE BROWN) STOCK INTO MICE OF THE VARIOUS LINES AND THEIR HYBRIDS

Group inoculated	Milk agent	Tumor #5687 D—line 2		Tumor #5736 D—line 1	
		+	−	+	−
D stock, line 2	D_2 stock	35	0	0	12
D stock, line 1	D_1 stock	0	38	6	0
ZbD_1F_1	None	0	15	15	0
ZbD_2F_1	None	11	0	0	11
ZzD_1F_1	Z(C3H) stock	0	8	8	0
ZzD_2F_1	Z(C3H) stock	6	0	0	6

		Tumor #6086 D—line 2		Tumor #6087 D—line 8		Tumor #6088 D—line 1	
		+	−	+	−	+	−
D stock, line 2	D_2 stock	33	0	0	33	0	30
D stock, line 8	D_8 stock	0	25	25	0	1	15
D stock, line 1	D_1 stock	0	10	6	4	9	1

TABLE II: INCIDENCES OF MAMMARY CANCER IN MICE OF DIFFERENT SUBLINES OF THE DILUTE BROWN (D) STOCK

	Non-breeding females				Breeding females			
			Average age in days				Average age in days	
Line	No.	% Ca.	Ca.	Non-ca.	No.	% Ca.	Ca.	Non-ca
D_1	45	51.1	504	642	50	82.0	366	378
D_2	88	59.1	504	600	134	69.4	387	420
D_8	50	66.0	459	633	62	77.4	405	438

considerable variation in the tumor data has been reported by Burrows (32) among the descendants of individual pairs of this strain when they were continued as sublines (Table III).

TABLE III: FROM BURROWS (32)—INCIDENCES IN LINES OF THE DILUTE BROWN STOCK DESCENDED FROM FIVE PAIRS

			Average age in days	
Line	No.	% Ca.	Cancer	Non-ca.
A	19	63	418	423
B	132	58	498	487
C	134	57	441	524
D	92	57	442	478
E	69	10	439	544

As indicated by the incidences of mammary tumors there are many sublines of the C57 black stock. While mice of one line may remain non-cancerous and not propagate the milk agent (Andervont, personal communication), incidences in excess of 75 per cent have been reported (39, 46, 10) for mice with the agent. A line of this stock may remain high cancerous for as many as 12 generations (10). Since it has not been shown that mice of the C57 black stock possess the inherited hormonal influence (30, 48, 29), to be discussed later, the propagation and transmission of the milk agent would become evident only in those studies in which breeding females had been observed. The variations in the incidences for other types of tumor are comparable to those for mammary cancer (61).

The relative role of the various causative factors in the genesis of spontaneous mammary cancer in mice is based upon the interpretation of the experimental data. Sublines have been found to exist within several of our most highly inbred stocks and these genetic differences may influence the incidence of spontaneous cancer. When results are obtained in different laboratories, environmental factors must also be considered as well as possible genetic differences in the animals. The relative importance of environmental versus genetic influences cannot be estimated unless control animals have been observed for each experimental series.

An excellent description of the value of controls has been given by Cannon (33):

"The Error of Omitted Control is another and inexcusable scientific misdemeanor. A control, in a scientific sense, is a standard of comparison used to check an inference drawn from an experiment. . . . There is little doubt that this experiment could be repeated many times and would always give the same result. *That* criterion of a 'good' experiment, therefore, is faulty; for, as long as the errors remain constant, their effects will reappear. Thus, the mere fact that a procedure can be repeated may not have any pertinence at all, because of the lack of corrective controls.

"A motto to be reverentially respected by every investigator who introduces new factors in his experiment and by the reader of reports of complex experimentation is *'cherchez le controle'*."

A poor control is worse than none at all for only those familiar with research in that particular field may realize that fact. Only the variable being tested should exist between the experimental and control animals. For example, mice that have been continued as virgins should never be used as controls for breeding mice of the same strain that have been subjected to another experimental variable. In such a case, the difference noted in the results may be the effect of the variation in hormonal stimulation and not the experimental agent or effect being tested.

Unless we employ homozygous animals in our research where we may "hold genetics constant while

we alter other factors such as hormonal level or other chemical or physiological variables" (59), the analysis of the results becomes even more difficult. Such an interpretation of data may be based upon standards established by the investigator and not upon the physiological standards of the experimental animal.

Since 1939 most investigators (20, 23, 26, 60, 74, 76) have accepted the theory that mammary cancer in mice generally results from three primary factors: inherited susceptibility, hormonal stimulation, and the mammary tumor milk agent, transferred by way of the milk (18). By controlling any one factor, the incidence of the disease may be greatly reduced. The most effective method, since it permits the strain to be maintained, is by the elimination of the milk agent by foster nursing. One line of fostered susceptible mice, started in 1934 (18), has been continued for over 30 generations with an incidence of approximately 1 per cent while in the cancerous unfostered line of the same stock there has been over 50 successive generations of mammary cancer and an incidence in excess of 90 per cent. If mice of the fostered line obtain the agent, either by nursing or the injection of extracts of tissues from mice with the agent, these mice may become cancerous and give rise to cancerous lines. (There is no evidence that the agent may be involved in the genesis of any other type of cancer in mice.)

In addition to the three causes mentioned above, another inherited factor has been described which operates through the genetic control of the hormonal mechanisms (30, 48, 29), termed the inherited hormonal influence (23). It may determine whether or not virgin females of susceptible strains with the agent will give rise to mammary cancer but its effects are not needed for the development of mammary cancer in breeders because of the increased hormonal stimulation associated with pregnancy.

By making reciprocal crosses between various inbred strains of mice, cancerous and low-cancerous, it has been possible to obtain preliminary observations on the genetic aspect of the various inherited factors, the role of the genetic constitution in the transmission of the milk agent, comparative studies on the milk agent as transferred by mice of different strains, and so forth. By the injection of extracts with the agent into susceptible mice without the agent, one may study the biological characteristics of the agent, its distribution in the body, and, as indicated by the results in the test animals, the relative activity of extracts of different tissues.

Even before the mammary tumor milk agent had been demonstrated, it was found that when mice of the cancerous D[1] and A strain were crossed, the reciprocal F_1 hybrids had, even when they were maintained as breeding females, incidences and average cancer ages correlated with their maternal strain (17, Table IV).

TABLE IV: INCIDENCES OBTAINED IN BREEDING FEMALES OF THE A AND D CANCEROUS STRAINS AND THEIR RECIPROCAL F_1 HYBRIDS

A ♀ — x — D ♂	D ♀ — x — A ♂
64%—12.3 mo.	56%—14.2 mo.
ADF_1	DAF_1
87%—12.1 mo.	72%—15.6 mo.

The cross between the high cancerous A and low cancerous B (C57 black) stocks gave results which demonstrated that when the reciprocal F_1 hybrids, with comparable genetic constitutions and hormonal stimuli, obtained the milk agent from the same (A) stock, approximately the same incidence of tumor may be observed in the two groups (18–21, 23, Table V). In order to secure high incidences of mammary cancer the females had to be used as breeders for the virgins of the susceptible stock and the hybrid generations gave low incidences (19). In the breeders the incidences were as high in the hybrids born to females of the low cancerous stock but nursed by females of the cancerous strain as when they had maternal parents from the cancerous strain and received the same milk agent.

TABLE V: DATA FOR MAMMARY CANCER IN HYBRID BREEDING FEMALES OF THE CROSS BETWEEN THE A AND B (C57 BLACK) STOCKS. ALL F_1 FEMALES WERE NURSED BY FEMALES OF THE A STRAIN; F_1 MOTHERS NURSED THEIR F_2 PROGENY, ETC., (21, 23)

Stock	No.	% Ca.	Average age in days	
			Ca.	Non-ca.
ABF_1	123	94.4	336	369
BAF_1	111	90.1	346	568
ABF_2	277	77.3	397	535
BAF_2	114	69.3	452	632
ABF_3	339	67.0	378	545
BAF_3	278	65.8	388	577

When Andervont (1) mated females of the C3H stock with males of the I and Y strains, variations were seen in the respective F_1 groups if the mice were kept as virgins; in the breeders there was only a slight difference in the average cancer ages (Table VI).

[1] Previously referred to as the dBr stock (81, 63), more recently as the dba or d strain (72, 73).

TABLE VI: INCIDENCES OBSERVED BY ANDERVONT (1) IN C3H MICE AND HYBRIDS PRODUCED BY MATING FEMALES OF THE CANCEROUS C3H STOCK WITH MALES OF OTHER STRAINS

Stock	Continued as:	No.	% Ca.	Average age in months Ca.	Non-ca.
C3H × C3H	Breeders	54	100	8.1	—
C3H × I	Breeders	25	96	8.5	17.5
	Non-breeders	20	90	11.4	17.0
C3H × Y	Breeders	31	94	10.7	21.0
	Non-breeders	24	79	12.7	19.9

Van Gulik and Korteweg (82) observed a high incidence (85 per cent) in virgin females of their subline of the dilute brown stock whereas those of the C57 black strain with the agent showed an incidence of 13 per cent (Table VII). Hybrids with

TABLE VII: INFLUENCE OF NURSING UPON THE INCIDENCE IN VIRGIN FEMALES—FROM VAN GULIK AND KORTEWEG (82)

Stock	Nursed by D ♀ No.	% Ca.	Nursed by B ♀ No.	% Ca.
D	168	85	110	9
B (C_{57})	67	13	194	0
DBF_1	335	69	47	4
BDF_1	42	46	722	2

the milk agent had high incidences whereas relatively low incidences were seen in mice nursed by females of the low cancerous strain. An analogous study in which the same parental strains were used was reported by Murray (71) in 1941 although some of the data had been previously published in 1935 from another laboratory by Murray and Little (72). During that interval the incidence had increased in virgin females of the dilute brown stock (Table VIII). Hybrids born of and nursed by mothers

TABLE VIII: DATA FOR VIRGIN FEMALES FROM MURRAY (71) IN 1941

Stock	Nursed by D ♀ No.	% Ca.	Nursed by B ♀ No.	% Ca.
D (dba)	297	50.8*		
	445	61.6	108	23.1
B (C57)	98	9.2	240	0.0*
D ♀ B ♂ F_1	113	39.8*		
B ♀ D ♂ F_1	93	64.5	379	6.1*

* Reported previously by Murray and Little (72) in 1935.

of the D stock showed an incidence of 40 per cent in 1935 whereas in the later study those with mothers of the C57 black stock but nursed by females of the cancerous strain had an incidence of 65 per cent.

Murray (70) also found that when dilute brown females were mated to males of the M (Marsh albino) cancerous strain the virgin F_1 females showed more tumors than did the reciprocal hybrids. However, quite the opposite was seen when females of the two cancerous strains were mated to males of the C57 black stock [2] (Table IX).

TABLE IX: INCIDENCES IN VARIOUS F_1 HYBRID GROUPS MAINTAINED AS NON-BREEDING FEMALES—FROM MURRAY (70)

Hybrids	No.	% Ca.	Ca. age mo.	Non-ca. age mo.
D ♀ M ♂ F_1	132	86	14.5	14.8
M ♀ D ♂ F_1	149	66	15.8	16.9
D ♀ B ♂ F_1	116	39	19.1	18.3
M ♀ B ♂ F_1	110	64	22.2	23.7

D = dilute brown; M = Marsh albino; B = C57 black stock.

Non-breeding females of the cancerous A strain have a low incidence whereas those of the C3H (Z) cancerous stock give a rather high incidence; breeders of each stock have high incidences. Observations obtained for the reciprocal F_1 and F_2 hybrids, kept as either virgins or breeders, are recorded in Table X (29, 30). In the breeding groups no significant difference was seen in either the females of the pure strains or hybrid generations while the virgin hybrids descended from mothers of the A strain had higher incidences and earlier average cancer ages than did those of the reciprocal generations. Heston and Andervont (48) made reciprocal matings between mice of the latter's C3H line and a subline of the same A strain and secured data for the F_1 virgin populations. They concluded that similar results were to be seen regardless of either the maternal or nursing strain (Table XI).

These two crosses between the A and C3H strains resulted in the theory involving the genetic control of the hormonal mechanisms or the inherited hormonal influence (23).

As shown in Table XII, first and second genera-

[2] It is possible that the DBF_1 data were from the 1935 report of Murray and Little (72).

TABLE X: OBSERVATIONS OBTAINED FOR VIRGIN AND BREEDING FEMALES OF THE A AND Z (C3H) STOCKS AND THEIR RECIPROCAL HYBRIDS (29, 30)

Stock	Breeding females No.	% Ca.	Aver. age in mos. Cancer	Non-ca.	Non-breeding females No.	% Ca.	Aver. age in mos. Cancer	Non-ca.
A	128	87	10.0	15.0	102	4	15.0	19.3
Z (C3H)	224	95	9.1	10.4	92	63	13.3	15.9
AZF_1	51	98	9.7	11.5	93	93	15.2	18.9
ZAF_1	40	98	10.1	7.5	56	73	19.1	22.5
AZF_2	46	96	11.2	8.5	98	70	16.2	21.8
ZAF_2	42	100	10.0	—	98	39	17.2	22.0

TABLE XI: FROM HESTON AND ANDERVONT (48)—DATA FOR NONBREEDERS

Stock	Nursed by:	No.	% Ca.	Aver. age in mos. Cancer	Non-ca.
A	A	22	0	—	16.4
A	C3H	32	16	10.6	16.9
C3H	C3H	29	93	10.9	14.0
C3H	A	35	94	11.5	17.5
A ♀ C3H ♂ F_1	A	45	91	15.8	21.3
C3H ♀ A ♂ F_1	C3H	36	83	13.8	14.3

tion descendants of C3H mice nursed by females of the A strain gave, when they were maintained as virgins, a higher incidence than did those with the C3H milk agent.

TABLE XII: MAMMARY CANCER IN VIRGIN FEMALES OF THE C3H STOCK WITH EITHER THE A OR C3H MILK AGENT

Milk agent from:	No.	% Ca.	Aver. age in days Cancer	Non-ca.
C3H stock	94*	63	399	477
C3H stock	33†	64	387	523
A stock	89†	80	407	500

* from (29); † observed during same period.

Warner and his associates (84) found that virgin F_1 females with mothers from the A strain gave a much higher incidence than did those with maternal parents of the cancerous Marsh albino strain in that reciprocal cross (Table XIII). Later Murray

TABLE XIII: INCIDENCES IN NON-BREEDING FEMALES OF THE A AND M (MARSH ALBINO) STOCKS AND THEIR HYBRIDS—WARNER *et al.* (84) AND (*), FROM MURRAY AND WARNER (73)

Stock	Incidence in mothers	No.	% Ca.	Ca. age
A		207	29.0	423
M		396	63.5	465
A ♀ M ♂ F_1		278	98.0	345
M ♀ A ♂ F_1		259	53.0	588
M ♀ A ♂ F_1 *	High	121	76.0	587
	Intermediate	69	50.7	642
	Low	61	14.8	568

and Warner (73) reported on sublines of the Marsh strain with different incidences, and a further tabulation of the MAF_1 data (84) showed that the results could be correlated with the incidence in the subline of the maternal parent (Table XIII). When the hybrids had mothers of the Marsh line with a low incidence but were nursed by females of either the A or dilute brown stocks, high incidences were obtained, while those nursed by either their maternal parents or females of the C57 black stock showed the incidence characteristic of those groups (Table XIV).

Because of the difference secured in the incidence in the reciprocal hybrids between the A and C3H stocks (Table X) and since a few hybrids with C3H mothers and D_2 fathers gave approximately the

TABLE XIV: INCIDENCES OF MAMMARY CANCER IN VIRGIN F_1 FEMALES PRODUCED BY MATING LOW MARSH FEMALES AND A MALES FOLLOWING FOSTER NURSING—FROM MURRAY AND WARNER (73)

Hybrids	Nursed by:	No.	% Ca.	Cancer age, days
MAF_1	A stock	35	85.7	364
MAF_1	D stock	27	81.5	551
MAF_1	M stock	43	16.3	572
MAF_1	B stock	28	0.0	—

same incidence as did the AZF_1 hybrids, reciprocal crosses have been made between the A, C3H (Z), and two (D_2 and D_8) sublines of the D or dilute brown strains. The preliminary results are tabulated in Table XV. Although many of the animals in

TABLE XV: INCIDENCES OF MAMMARY CANCER IN VIRGIN FEMALES. IN THE HYBRID GROUPS THE FIRST CAPITAL LETTER DENOTES THE MATERNAL STOCK AND THE SMALL LETTER THE SOURCE OF THE MILK AGENT; IF OMITTED, THE MICE NURSED FEMALES OF THE MATERNAL STOCK

Group	No.	% Ca.	Average ca. age	Non-ca. age or % living	Born between*
Zz	127	63	396	489	6/45–3/46
Za	89	80	407	500	3/46–9/46
Aa	110	4	450	590	3/45
$ZzAF_1$	59	73	573	678	3/46–1/47
$ZaAF_1$	121	60	487	32%	3/46–1/47
$AaZF_1$	118	92	471	633	10/45–12/45
ZD_2F_1	105	93	351	1%	1/45–12/46
D_2ZF_1	79	70	496	19%	6/46–1/47
ZD_8F_1	89	94	339	3%	10/46–3/47
D_8ZF_1	50	40	387	58%	10/46–1/47
$D_2D_8F_1$	32	13	390	81%	1/47–5/47
$D_8D_2F_1$	73	21	441	74%	10/46–7/47
AD_8F_1	97	26	419	71%	9/46–5/47
D_8AF_1	30	7	450	90%	12/46–7/47
AD_2F_1	67	52	462	39%	8/46–5/47
D_2AF_1	40	13	471	85%	9/46–5/47

* From unpublished data.

certain groups are still living, the incidences correlated with the cancer ages and dates of birth may give some indication of what may be expected in the nature of complete data. ZDF_1 hybrids have approximately the same incidence as did AZF_1 virgins but their tumors appeared several months earlier. As suggested by the cancer ages, the AD hybrids will have a lower incidence than the AZF_1 animals. When females of the dilute brown stock were mated to males of the C3H strain, higher incidences are being obtained in the F_1 nulliparous females than when D females were crossed with males of the A strain. From the preliminary data it seems probable that the hybrids derived from the AD cross will show a lower incidence than was found when females of the same maternal stock were mated with C3H males.

Forty-two ZD_2F_1 breeding females were found to have an incidence of 76 per cent. The average cancer age was 290 days while the non-cancerous mice

survived to 362 days, an age later than the cancer age of the comparable F_1 virgin population.

Reference has been made to several studies in which the incidence of mammary cancer did not remain constant in inbred strains over a period of years, thus probably influencing the results observed in reciprocal crosses between different strains with either low or high incidences. Furthermore, when two or more cancerous strains are crossed the results cannot be predicted, for the incidences are not always comparable in the first hybrid generations, especially when the mice have been continued as non-breeders where two inherited factors, namely the inherited susceptibility and the inherited hormonal influence, are needed for the development of mammary cancer. Because the milk agent from different cancerous strains may not give the same incidences in mice with the same genetic constitution, the results might suggest that the agents may not be the same.

The interpretation of these experimental data as regards the activity and/or concentration of the milk agent becomes even more difficult when we consider the results seen following the injection of an extract with the agent in serial dilution studies. One such experiment, in which spontaneous mammary cancer was the source material, is tabulated in Table XVI. The tissue was extracted with distilled water and cleared by centrifugation at a speed of approximately 2,500 r.p.m. for 10 minutes. The test animals in this and other studies referred to in this report were ZBC hybrids, derived from a cross between the fostered A and C3H lines, in which the incidence in over 700 controls has been 1.5 per cent. Each mouse received, by intraperitoneal injection, from 10^{-1} to 10^{-6} gram equivalents of the source material suspended in 1 cc. The highest incidence was recorded in the mice of the group which received 10^{-3} gram equivalents while the earliest cancer age was seen in those which had been injected with the extract diluted ten-thousand-fold.

TABLE XVI: ACTIVITY OF THE MILK AGENT IN SPONTANEOUS MAMMARY TUMORS FROM MICE OF THE A STOCK WHEN EXTRACTED WITH DISTILLED WATER IN SERIAL DILUTION STUDIES

Gm. Equiv. injected	Age when injected	No.*	% Ca.	Average ca. age	No.**	% Ca.
10^{-1}	24—42	56	63	418	49	71
10^{-3}	30—40	29	69	407	24	83
10^{-4}	22—34	57	61	363	49	71
10^{-5}	22—32	27	41	420	15	73
10^{-6}	22—32	56	36	480	49	41

* Including non-cancer mice living to age of first tumor; ** living to average cancer age.

The activity of the mammary tumor milk agent in extracts of spontaneous mammary tumors which had developed in fostered mice of the C57 black stock is presented in Table XVII (27). Two of the tumors, No. 7476 and No. 7477, appeared in the offspring of a fostered female. These tumors were transplanted into mice of the same stock which had not been infected with the milk agent. The tests (Table XVIII) showed that the agent survived for 10 passages and indicated that the agent was at least as "active" and possibly more "concentrated" in the transplanted than in the original tumor.

Some of the properties of the milk agent in spontaneous mammary cancer were studied by means of centrifugation by Barnum, Ball, and Bittner (11). Three resuspended sedimentable fractions together with the initial and final supernatant fluids were tested in dilution ranging from 10^{-3} to 10^{-5} gram equivalents. Essentially all of the agent was sedimentable at 23,000 times gravity for a period of 1 hour and most of the activity was associated with the fractions containing the large particles and the microsomes of the cytoplasm (Table XIX).

In another experiment, Barnum, Huseby, and Bittner (12, Table XX) observed that "when a microsome fraction containing the milk agent is extracted at pH 10 and nearly all of the nucleoprotein is removed, the agent remains associated with the

TABLE XVII: ACTIVITY OF THE MILK AGENT IN EXTRACTS OF SPONTANEOUS MAMMARY TUMORS FROM C57 BLACK MICE, LINE 6. THE C57 MICE OBTAINED THE AGENT FROM EITHER A OR Z (C3H) FOSTER MOTHERS; TWO OF THE TUMORS AROSE IN FOSTERED ANIMALS; TWO IN THE PROGENY (A_2) OF FOSTERED MICE

Tumor no.	Date injected	Gm. Eq. injected	Age of mice when inj.	No.	% Ca.	Aver. ca. age	Av. non-ca. age or % living
7476 (A_2)*	6/27/46	1/50	20—27 Days	20	80	455	609
	"	1/1000	20—27 "	13	46	429	531
7477 (A_2)*	"	1/50	20—25 "	29	59	373	456
	"	1/1000	20—27 "	25	80	486	4%
7478 (Z_1)	"	1/100	18—22 "	32	78	444	3%
7788 (Z_1)	3/26/47	1/50	22—25 "	56	71	314	25%
	"	1/1000	22—25 "	33	73	315	24%

* Tumors inoculated.

TABLE XVIII: COMPARATIVE ACTIVITY OF THE MILK AGENT IN EXTRACTS OF MAMMARY TUMORS FROM C57 BLACK MICE. SPONTANEOUS TUMORS AND TRANSPLANTED TUMORS, AFTER ONE OR TEN PASSAGES IN MICE WITHOUT THE AGENT, WERE TESTED

Tissue extracted	Gm. Eq. injected	Tumor no. 7476 No.	% Ca.	Aver. ca. age	Av.non-ca. age or % living	Tumor no. 7477 No.	% Ca.	Aver. ca. age	Av. non-ca. age or % living
Spontaneous tumor	1/50	20	80	455	609	29	59	373	456
6/27/46	1/1000	13	46	429	531	25	80	486	4%
After 1 passage	1/50	9	78	420	537	29	100	371	—
7/23/46	1/1000	21	52	450	506	20	80	457	5%
After 10 passages	1/10	38	68	371	29%	38	79	317	16%
1/6/47 or 1/8/47	1/1000	44	41	360	52%	44	32	398	55%

'lipoprotein' portion and does not appear to have lost any of its activity. This does not imply that the milk agent contains no nucleic acid but rather that at least 95 per cent of the ribonucleic acid of the microsome fraction can be removed without impairing the activity of the agent." The supernatant fluids of the fractions treated at pH 9 and pH 10 showed little activity.

TABLE XIX: ASSAY OF THE MILK AGENT IN EXTRACTS OF SPONTANEOUS MAMMARY CANCER FROM *A* MICE IN CENTRIFUGAL FRACTIONS—FROM BARNUM, BALL, AND BITTNER (11)

Fraction	Gram Equiv. injected	No.	% Ca.	Average age in days
Sup. 8′, 500 g.	10^{-3}—10^{-5}	25	36	305
Sed. 5′, 23,000 g.	"	18	56	425
Sed. 1 hr., 23,000 g.	"	23	65	330
Sed. 1 hr., 70,000 g.	"	22	14	554
Sup. 1 hr., 70,000 g.	"	19	5	618

Extracts of either spontaneous or transplanted mammary cancer will stimulate the production of antibodies in both rats and rabbits and these will neutralize the agent *in vitro* and *in vivo* (4, 44, 45). That the antigen probably was the milk agent was indicated by the serological investigations of Gorodilova and Shabad (43), Bennision (13), and Imagawa, Green and Halvorson (52).

TABLE XX: STABILITY OF THE MILK AGENT IN BUFFERS AT pH 9 AND pH 10: FROM BARNUM, HUSEBY, AND BITTNER (12)

Fraction	Gm. Equiv. injected	No.	% Ca.	Aver. ca. age in days	% living
Large granules—	10^{-3} to 10^{-5}	26	65	279	19
Microsome pellet—pH 7	10^{-3} to 10^{-5}	25	84	292	16
Whole microsome—pH 9	10^{-3} to 10^{-5}	27	85	273	7
Microsome pellet—pH 9	10^{-3} to 10^{-5}	27	78	260	15
Whole microsomes—pH 10	10^{-3} to 10^{-5}	27	89	257	0
Microsome pellet—pH 10	10^{-3} to 10^{-5}	25	92	262	8

If mice with the milk agent are injected with breast cancer anti-serum produced in rabbits, we have been unable to prevent the development of mammary cancer. Twenty-seven C3H females, 26 to 46 days of age when first treated, were given at 12 day intervals three injections of 0.33 cc. of the antiserum. Many died young but including the non-cancerous mice which survived to the average cancer age, 82 per cent gave rise to mammary cancer. The incidence in the progeny (ZAF_1 hybrids) of 4 females was 85 per cent. Fifteen other C3H females received a single injection of 0.5 cc. of the antiserum when they were from 30 to 37 days of age; 92 per cent died cancerous.

Thus, the mammary tumor milk agent has the characteristics of an infectious agent or "virus," since:

1. It increases in amount in the presence of living cells.
2. It is macromolecular in size as shown by centrifugation.
3. It is specifically antigenic.
4. It remains active following lyophilization, dessication, and filtration.
5. It appears to have a rather wide range of pH stability.

For the past ten years, since the reports of Bagg (6–9), it has been assumed that "force breeding," rapid breeding with the prevention of nursing, would result in the acceleration in the time of development and increase the incidence of mammary cancer regardless of the fact that Murray (69, 31) found little difference in the incidence among the females of the dilute brown stock which nursed all or none of their litters. The effects of force breeding in C57 mice without the agent could not be confirmed by Little and Pearsons (62), Fekete (37), as well as by Bagg (7, 9). However, when mice of the C57 black stock obtain the milk agent, either low or high incidences may be obtained depending upon the subline. These facts make it probable that the foster mothers Bagg used to raise the mice in the original study and which later developed mammary cancer, probably transferred the milk agent; mice of that line have remained high cancerous (76 to 89 per cent) for 12 generations (10).

Bagg and Hagopian (9) assumed that with the prevention of nursing there was retention of milk because of improper drainage, which produced an

irritating chemical effect upon the epithelium of the mammary gland. Although Fekete and Green (38) reported that more tumors developed in blocked than in normal secreting glands of dilute brown mice, they stated that while the secreting function of the occluded glands was found to be normal, premature regression had occurred.

In the only study where normal nursing females and force breeders have been compared, Bagg (7) was unable to find any significant difference in their incidences. Similar results were obtained by us in some studies on the activity of the milk agent (25), and because of these findings, it seemed advisable to check the influence of force breeding versus normal breeding and lactation upon the development of mammary cancer in females of the cancerous dilute brown and C3H stocks. Also, because of our experience in obtaining lactating mammary tissue for studies on the milk agent, another group of C3H females was included where the mice were permitted to nurse their progeny for 12 days after which they were returned to the breeding pen.

The incidences of mammary cancer were found to be lower in mice of every subline of the D stock than were observed in normal nursing females and there was no acceleration in the time of onset (Table XXI). Also, in the C3H stock the mice subjected to

TABLE XXI: COMPARISON, BY SUBLINE, OF THE INCIDENCES AND AVERAGE AGES OF MAMMARY CANCER IN NORMAL AND FORCE BREEDERS OF THE DILUTE BROWN (D) STOCK

Subline	Breeding:	No.	% Ca.	Aver. age in days Cancer	Non-ca.
Line 1	Normal	50	82	366	378
Line 1	Force	24	67	451	362
Line 2	Normal	134	69	387	420
Line 2	Force	34	56	403	419
Line 8	Normal	62	77	405	438
Line 8	Force	19	63	399	433

force breeding showed lower incidences than did those which were permitted to nurse their progeny. In one group of 30 mice the time of development was delayed while in the second series of 48 mice it had been accelerated (Table XXII). The controls for the two studies gave the same incidences and average cancer ages. The females that were allowed to nurse for 12 days had the lowest incidence of any group and had approximately the same average cancer age as did the normal breeders.

To determine the relative amount of stagnation in the mammary glands of females which were not permitted to nurse their young (force breeders) and those which nursed for 12 days, females were sacrificed 24 hours after their young had been removed. Figs. 1 and 2 demonstrate the gross appearance of

TABLE XXII: INFLUENCE OF NURSING UPON THE DEVELOPMENT OF MAMMARY CANCER IN C3H FEMALES. MICE OF EACH GROUP WERE OBSERVED DURING THE SAME PERIOD

Breeding	No.	% Ca.	Aver. age in days Cancer	Non-ca.
Force	30	80	310	365
Normal	54	94	272	269
Force	48	81	234	271
Nursed for 12 days	47	74	263	287
Normal	87	93	270	297

their mammary glands. There was not much secretion of milk or stagnation in the glands of the force breeders while those that nursed for 12 days showed an accumulation of stagnated milk. Many of the latter group had engorged glands for several days and a few had to be killed or died as a result. The histological examination of sections of the glands by Dr. R. A. Huseby confirmed the gross findings.

These results would indicate that stagnation *per se* does not appear to be an important contributing cause of mammary cancer in mice.

Lathrop and Loeb (53), Loeb (64), Cori (35), and Murray (67–68) were in agreement as to the effect of ovariectomy upon the genesis of mammary cancer in mice and concluded that the younger the females were when their ovaries were excised the lower the incidence would be. When Woolley, Fekete, and Little (85–87, 39–40) operated upon day-old mice of the dilute brown stock, they observed, instead of a low incidence, that the same percentage of the excised mice developed mammary cancer as they found in intact virgin females of that strain. They noticed that castrated females of the dilute brown and C3H stocks showed evidence of estrogenic stimulation and were able to demonstrate that the source of the hormones was the adrenal cortex which had become hyperplastic. Operated females of the C57 black stock did not show either the characteristic adrenal alterations or stimulation of the secondary sex organs (86).

The same investigators (88–94) observed adrenal carcinoma, in addition to adrenal hyperplasia, in castrated animals of the Ce (extreme dilution) [3] stock. In castrated females the submaxillary glands were essentially of the female type for the first 5 months but following that age they corresponded to the type seen in young intact males. Evidence for both estrogenic and androgenic effects occurred simultaneously in the same animal. In castrated males the type of submaxillary gland might be male, female, or intermediate.

One adrenal cortical carcinoma that developed

[3] Probably developed by Strong (79) as the extreme dilution or E stock.

in an ovariectomized female of the Ce strain was transplanted by Woolley and his associates (94). The host had a male type of submaxillary gland and usually the transplanted tumors showed signs of having an androgenic influence upon the host. In 3 intact females, one with an abdominal transplant had a submaxillary gland of the male type while 2 with subcutaneous growths had the female type.

Hybrids with A mothers and Ce fathers showed, when castrated, the same physiological effects seen in animals of the parental Ce stock; that is adrenal carcinoma (Huseby and Bittner, unpublished).

Ovariectomized females of the I strain, secured from Andervont, and AIF_1 hybrids will develop hyperplastic adrenals and show estrogenic stimulation of the uteri and vaginal mucosa (49).

In mice of the C3H strain and hybrids between the A and C3H stocks an association has been found between the inherited hormonal influence and hyperplasia of the adrenal cortex in ovariectomized females (77, 78). To secure further data on this possible relationship, females of the A strain, that have a low incidence as virgins (19, 29) and do not show the typical adrenal changes following castration (77, 78), were mated to males of the D and I stocks as well as males of the Ce strain. The data on the development of mammary cancer in the respective F_1 groups kept as virgins are presented in Table XXIII. Included for comparison are published and unpublished observations on the A and Z (29) and A × B or C57 black (19) crosses as similar results were obtained in these series.

TABLE XXIII: INCIDENCES OF MAMMARY CANCER OBSERVED IN VIRGIN F_1 FEMALES PRODUCED BY MATING FEMALES OF THE A STRAIN WITH MALES OF VARIOUS STRAINS. WITH THE EXCEPTION OF THE B MALES, THE PATERNAL PARENTS WERE FROM STOCKS WHICH SHOWED ADRENAL ALTERATIONS FOLLOWING CASTRATION. VIRGIN A FEMALES HAVE A LOW INCIDENCE (TABLE 15)

Group	No.	% Ca.	Average Ca. age	Non-ca. age or % living	Born* between
AZF_1	118	92	471	633	10/45–12/45
AD_8F_1	97	26	419	71%	9/46–5/47
AD_2F_1	67	52	462	39%	8/46–5/47
AIF_1	63	44	417	46%	9/45–3/47
$ACeF_1$	70	29	510	47%	4/46–12/46
ABF_1	125	4	657	768	6/45–11/45

* from unpublished data.

The preliminary results for some groups and final data for others demonstrate that when females of the A strain are mated to males of strains which develop adrenal hyperplasia following castration, the F_1 virgins have a greatly increased incidence of mammary cancer. Hybrids between the A and B stocks have low incidences when kept as nonbreeders and neither strain show the castration effects. While an increased incidence was noted in the $ACeF_1$ virgins, it was lower than was recorded in the AZF_1, ADF_1, and AIF_1 hybrids.

Some results are available on the incidences of mammary cancer in breeding females of different groups and they might be mentioned at this time although some of the numbers are too small to be of great importance. Seventeen AD_2F_1 breeding females were continued and the majority of them subjected to force breeding. Of these, 3 are still living while the others have developed mammary cancer at an average age of 361 days. Only 8 AIF_1 breeders were maintained; all died cancerous at an average age of 215 days. Ten of the 35 $ACeF_1$ females are still under observation but 65 per cent of the total have developed cancer at an average age of 351 days.

Observations are available for a small number of Ce mice which had obtained the milk agent by foster nursing. Four mice of the fostered generation, although they were mated, failed to have progeny and died noncancerous. Eleven breeders of the fostered generation have developed mammary cancer at an average age of 543 days; in fact, no animal which had offspring has died noncancerous. This included one depleted litter of 5 females. Tumors have appeared in about 15 of the first and second generation descendants of the fostered Ce mice. From preliminary data it seems probable that hybrids with these Ce mothers and C3H fathers will have high incidences in both virgin and breeding females. The tumors are appearing at an earlier average age in the hybrids than they did in the females of the Ce stock.

Data cannot be given on the incidence of mammary cancer in mice of the I strain when they have the milk agent. However, Andervont (2) reported that a low incidence was secured, suggesting that the animals may have a low susceptibility for such tumors.

Ovariectomized females of the NH strain (42, 41), as well as intact females since they undergo physiological castration (41), may also give rise to adrenal carcinoma. In ovariectomized females of the C3H stock the type of hormones secreted by the altered adrenals may be modified by diet (34).

In an attempt to determine how the inherited hormonal influence might function, ovaries of females of the A and C3H stocks and their reciprocal F_1 hybrids were transplanted into spayed F_1 animals (49–51). "By this procedure the grafted ovaries would be under similar or identical pituitary stimulation and the hormones produced by them

TABLE XXIV: INCIDENCES OF MAMMARY CANCER AND CANCER AGES OBSERVED IN BREEDING AND VIRGIN FEMALES AND IN CASTRATED ANIMALS WITH TRANSPLANTED OVARIES—FROM HUSEBY AND BITTNER (49)

Stock	Non-breeding ♀ ♀ % Ca.	Non-breeding ♀ ♀ Age	Breeding ♀ ♀ % Ca.	Breeding ♀ ♀ Age	Ovariectomized females with transplanted ovaries	% Ca.	Age
A	4	450	92	300	A with A ovaries	5	378
Z(C3H)	63	399	95	273	Z with Z ovaries	67	384
$AZF_1 + ZAF_1$	83	515	98	297	F_1 with F_1 ovaries	70	531
					F_1 with A ovaries	57	603
					F_1 with Z ovaries	66	495

F_1 castrated males with transplanted F_1 ovaries	
% Ca.	Age
87	345

would be metabolized in the same manner and would act upon the same end organs. Thus any difference in the incidences of mammary cancer would, in all probability, be attributable to a difference in ovarian secretion *per se*" (49). Differences noted in the vaginal smears of the various groups, as well as details of microscopic findings of the pertinent material, will be found in the original manuscript. The data for mammary cancer (Table XXIV) would indicate that some of the physiological effects of the inherited hormonal influence are mediated through an inherited ovarian function as well as other organs of the endocrine system. The hormonal stimulation from the grafted ovaries prevented adrenal alterations in the ovariectomized animals. Castrated F_1 males with transplanted ovaries gave rise to a high incidence of mammary cancer and they developed nearly 6 months earlier than in the virgin females of the same generation.

In virgin females of the A strain the incidence of mammary cancer may be increased by the transplantation of pituitary grafts (65, 66).

In summary we may state that the inherited hormonal influence is determined by some genes which are not involved in the determination of the inherited susceptibility for spontaneous mammary cancer. The inherited hormonal influence has been found to be transmitted by several strains of susceptible mice which have a high incidence in virgin females; one stock may have a low susceptibility and transmit the hormonal factor. Some of the physiological effects of the hormonal influence are mediated through ovarian activity, as well as other organs of the endocrine system. Difference in the initiation of estrus has also been noted (36). In several strains which show hyperplasia of the adrenal cortex following castration, an association has been found between the inherited hormonal influence and the adrenal changes. Although mice of the Ce stock may show a high incidence of mammary cancer when they have the milk agent, further studies must be completed before it will be possible to state whether or not the hormonal factor which they transmit, characterized by adrenal carcinoma in castrated animals, may be comparable to the inherited hormonal influence.

Following the removal of the gonads, the physiological response of the animals of various strains is determined by genetic factors which are transmitted. Further study may demonstrate several of these inherited hormonal factors.

Preliminary data on the excretion of unconjugated ketosteroids in the feces of mice of the same stock with and without the milk agent, from Samuels and Bittner (75, 28), are reported in Table XXV.

TABLE XXV: ASSAYS ON UNCONJUGATED 17-KETOSTEROIDS IN THE FECES OF MICE OF THE SAME STOCKS WITH AND WITHOUT THE MILK AGENT—FROM SAMUELS AND BITTNER (75)

Strain	Milk agent	Free ketosteroids γ/100 mouse, days	
Z (C3H)	By nursing	30	
Zb (C3H)	Absent		56
A	By nursing	<20	
Ax	Absent		74
C3H	By nursing	74	
C3H	Absent		240
D+	By nursing	<20	
D	By nursing	<20	
AZF_1	By nursing	<20	
$AxZbF_1$	Absent		48
"	Inj. 10^{-1} gm. equiv.	<20	
"	Inj. 10^{-2} gm. equiv.	<20	
"	Inj. 10^{-3} gm. equiv.	<20	
C57 blk.	Absent		235

In every instance higher readings were obtained for mice of the same strain without the milk agent. The excretion rates for the hybrids between fostered A and C3H lines without the agent were altered, following the injection of extracts with the agent, to that found for mice of the same generation which showed a high incidence of mammary cancer. The data for the controls, injected with the same extracts and maintained in Minnesota, are reported in Table XXVI.

The variation obtained in the excretion rates for mice of different cancerous stocks may be of importance but cannot be correlated directly with the incidences of mammary tumors in either virgin or breeding females or with any known inherited characteristic.

TABLE XXVI: INCIDENCE OF MAMMARY CANCER OBSERVED IN CONTROL ZBC MICE, INJECTED WITH EXTRACTS REFERRED TO IN TABLE 25, AND MAINTAINED IN MINNESOTA

Age of mice when injected, days	G. Eq. injected	No.	% Ca.	Average Ca. age	% Living
23—25	10^{-1}	20	70	373	5
23—25	10^{-2}	20	65	376	10
23—25	10^{-3}	20	85	327	5
	Incidence among the progeny of injected mice.				
	10^{-1}	17	65	317	24
	10^{-3}	21	90	298	5

In some strains and their hybrids those with the milk agent came into estrus more often than when the mice were not so infected with the agent (49). However, the results were not consistent for all the groups and further studies must be completed before the significance of these data may be apparent.

These results will form the basis for further research to ascertain if the role of the milk agent in the genesis of mammary cancer in mice may be to alter the hormonal metabolism or production of the host; whether or not mice with the agent may have more hormones with "carcinogenic" activity than do mice of the same stock without the agent. If so, the organ in which this would be expected to occur probably would not be the mammary gland. Although the mammary tumor milk agent has been shown to be active only in the development of mammary tumors, it is distributed widely throughout the body. The high concentration in the mammary glands may be responsible for the propagation of the agent to animals of the next generation and for the survival of the agent.

The long latent period between the introduction of the milk "virus" and the development of mammary cancer in mice would be understandable if the hormones, altered because of the action of the "virus," might be the "primary" cause although the interaction of all the causative factors would be prerequisites for a high incidence.

SUMMARY

The interpretation of data on the genesis of mammary cancer in mice, where the disease results from the interaction of several causative factors, is becoming more difficult as shown by a review of the results of various experiments.

Because of the isolation of sublines within inbred strains of mice, the possible influence of the altered genetic constitutions as well as environmental factors upon the final results, controls should be maintained for each experimental series.

The physiological response of animals of various stocks following castration has been found to be dependent upon the genetic constitution of the animals. In several strains an association has been found between adrenal hyperplasia, with estrogenic stimulation of the secondary sex organs, and the inherited hormonal influence. The possibility of other inherited hormonal factors is discussed.

As the basis for further research, it is suggested that the role of the mammary tumor milk agent in the genesis of mammary cancer in mice may be to alter hormone metabolism with the production of more "carcinogenic" hormones.

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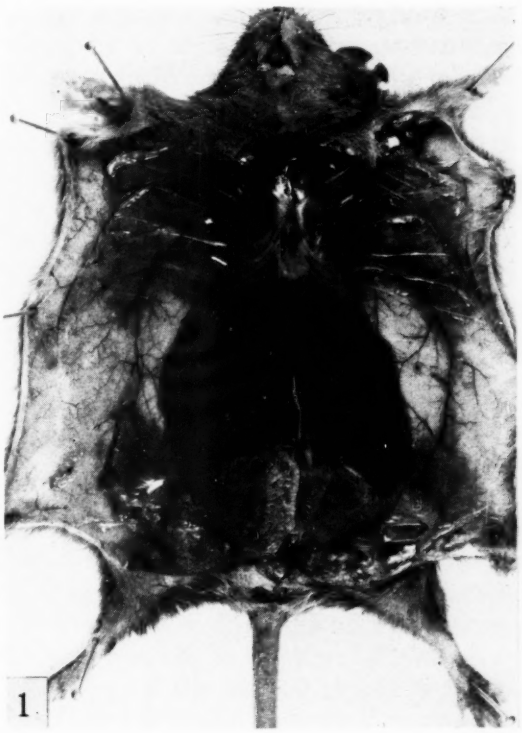

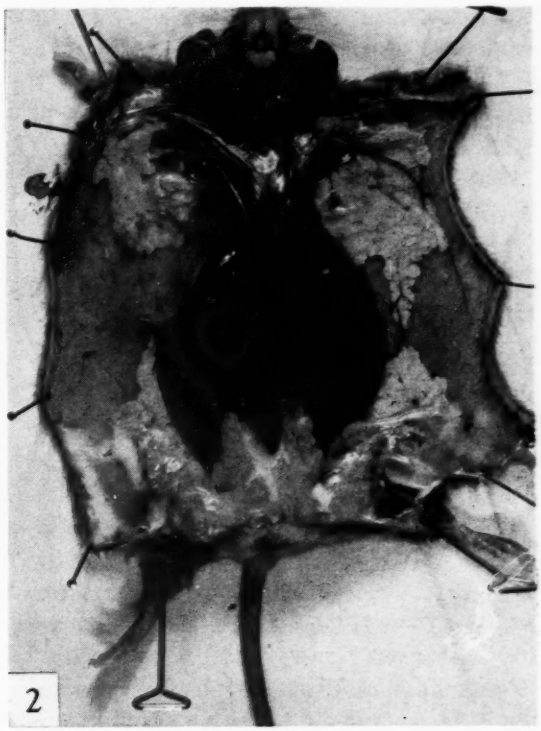

FIG. 1.—Gross appearance of mammary glands of a force bred C3H female 24 hours following removal of the young.

FIG. 2.—Mammary glands of a C3H female that nursed her progeny for 12 days. The dissection was made 24 hours after the young had been removed.

The Relationship of the Inherited Hormonal Influence to the Production of Adrenal Cortical Tumors by Castration*

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The work of Loeb (21, 22), Cori (6, 9) and Murray (23, 24) demonstrated that ovariectomy of mice in certain stocks at an early age was associated with a reduction in incidence of spontaneous mammary tumors. Hyperplasia and/or adenoma of the adrenal cortical cells in castrated male and female mice was later described in several inbred stocks (dba, ce, C3H, CBA, NH, Balb) (13, 14, 27, 29). The altered adrenal cortical cells in the castrated mice were linked with gross and histological evidences of estrogenic stimulation of the mammary glands, uterus and vagina. Dorfman and Gardner (10) supported the evidence of physiological activity of adrenal tumors in studies upon ovariectomized mice of the NH strain in which the urinary output of estrogenic substances was increased approximately 4 times over that found in the intact female. Transplants of adrenal tumors (32) and the structure of the submaxillary gland (30) in castrated mice of the extreme dilution stock demonstrated that secretions from the adrenal lesion also contained compounds with androgenic activity.

The presence of the adrenal lesions in castrated mice was not correlated with the occurrence of mammary tumors in the stocks mentioned above, in spite of the fact that the original observation of the adrenal lesion occurred coincidentally with the unexpected appearance of mammary tumors in castrated mice (27). The development of mammary tumors in mice is known to be influenced by 3 factors including (a) the genetic susceptibility of the animal, (b) the presence in the milk of the nursing female of an agent transmissible to the offspring, and (c) adequate hormonal stimulation of the mammary gland (3). The tumors in the adrenal cortex were observed in castrated animals of those strains (27, 28) in which a high incidence of spontaneous mammary cancer had been established and associated with the presence of the three factors (2, 4, 6), and had also been observed in low mammary tumor stocks as well (14, 28). Because all stocks have not been completely tested for the presence of the 3 factors influencing the growth of mammary tumors, the importance of these 3 factors in the development of adrenal tumors was studied in the present experiment.

The genetic nature of 2 of these factors, the hormonal mechanism and the mammary tumor susceptibility, was suggested by Bittner and his co-workers (7). Female mice of the high mammary tumor A and C3H stocks and reciprocal hybrids between the 2 stocks were studied. In the 2 inbred strains, a high incidence of mammary tumors was observed among the breeding animals. If the mice were maintained as virgins, the incidence of mammary tumors was high in the C3H stock but low in the A strain (4, 7). The genetic susceptibility for mammary tumor development and an active agent in the milk were present in mice of both groups. Precancerous nodules and tumors of the mammary glands were found consistently in virgin mice of the C3H and hybrid stocks but were rarely present in mice of the A strain.

The difference in mammary gland structure and mammary tumor incidence between the virgin mice of the A strain, and virgin mice of the C3H and hybrid strains was explained on the supposition that "all three of the primary factors (3) responsible for mammary tumor development must be present in order for nodules to develop with any frequency," and in the virgin mice of the A strain there was inadequate hormonal stimulation (6). The effective hormonal mechanisms as measured by the mammary tumor incidence was thought to be a dominant genetic factor transmitted from the C3H stock to hybrid animals between the A and the C3H stocks of the F_1 and F_2 generation, and was termed the inherited hormonal influence (7).

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The hormonal difference in genetic complex influencing mammary tumor formation in these 2 strains and in the F_1 hybrids was confirmed by Heston and Andervont (17).

To examine the action of the inherited hormonal influence, castration of mice in the A and C3H strains was suggested to determine what relationship might exist between the inherited hormonal factor, mammary tumor development and adrenal cortical hyperplasia. Preliminary evidence has been reported that the inherited hormonal influence as it governs hormonal metabolism may be associated with the adrenal cortical hyperplasia in castrated mice of the A and C3H stocks and their hybrids (25). Final data will be presented at this time.

MATERIALS AND METHODS

Intact and castrated females of several groups of animals were studied: mice of 2 high mammary tumors strains (A and C3H), and 6 groups of reciprocal hybrids between these 2 stocks (4 with the active milk agent (AZF_1, ZAF_1, AZ–ZBC, ZA –ZBC) and 2 without this agent (AxZb–ZBC, ZbAx –ZBC). The mice were derived from the Bittner colony.

The breeding female mice in both the A and the C3H stocks have a high incidence of mammary tumors. Most of the virgin female mice of the C3H stock also had mammary tumors. The virgin mice of the A strain rarely had mammary tumors (Table I).

The reciprocal hybrid mice were separated into 2 groups: one with a high incidence of mammary tumors and the other with a low incidence of mammary tumors. The high tumor hybrids (AZF_1, ZAF_1, AZ–ZBC, ZA–ZBC) were offspring resulting from the reciprocal cross between the A and the C3H (Z) strains, and the mating of these offspring with C3H males. The animals in these groups have a high incidence of mammary tumors comparable to that observed among breeding female mice in the inbred strains from which they were derived (Table I), (5).

The low tumor hybrids (AxZb–ZBC, ZbAx–ZBC) were derived by mating offspring of reciprocal crosses between fostered mice of the A and C3H strains with males of the C3H strain. In this manner hybrids were obtained with the genetic susceptibility and hormonal mechanisms of the parent strains but were lacking an active milk agent. The tumor incidence in these fostered mice has been 1 to 2 per cent (5).

The 3 factors known to influence the production of mammary tumors in mice were present in the following groups: (a) breeding and virgin female mice of the C3H stock; (b) the F_1 and F_2 hybrid mice between the A and the C3H stock; (c) the hybrid animals derived from the backcross to the C3H males, and (d) breeding females only of the A strain. The hormonal mechanism was lacking in the virgin mice of the A strain. The milk agent was not present in the groups of fostered mice.

All animals were castrated at 4 weeks of age. Both the intact and the castrated mice were checked at weekly intervals for the appearance of mammary tumors. Four months after ovariectomy vaginal smears were taken at intervals of 4 to 6 weeks to determine the presence of vaginal cornification. The cotton swab technic was used. A few animals with vaginal cornification were selected at random from each group and were smeared daily for a 2 week period to determine whether or not cyclic variations occurred. The smearing was discontinued when the animals were 18 months old. Smears were considered positive and indicative of estrus only when cornified epithelial cells were the chief cellular constituent of the smear.

All animals that acquired mammary tumors were killed shortly after the nodule was noted. Mice in poor condition were also sacrificed for study. Tisues removed at autopsy were fixed in either Bouin's or Helly's fluid, and were stained with Delafield's hematoxylin and eosin. Whole mounts of the second and third pair of mammary glands, stained with Mayer's hemalum, were also prepared.

The animals were housed in wooden boxes, 5 or 6 in a group, with an unlimited supply of Purina

TABLE I: THE INCIDENCE OF MAMMARY CANCER IN BREEDING AND VIRGIN FEMALE MICE OF THE A AND THE C3H STOCK, AND IN HYBRID ANIMALS BETWEEN THESE TWO STOCKS*

Strain	Breeding			Virgin		
	% Ca.	Av. ca. age	Av. non-ca. age	% Ca.	Av. ca. age	Av. non-ca. age
A	86.7	10.0 mo.	15.0 mo.	3.9	15.0 mo.	19.3 mo.
C3H(Z)	95.1	9.1	10.4	63.0	13.3	15.9
AZF_1	98.0	9.7	11.5	92.5	15.2	18.9
ZAF_1	97.6	10.1	7.5	73.2	19.1	22.5
AZF_2	95.8	11.2	8.5	70.4	16.2	21.8
ZAF_2	100.0	10.0	—	38.8	17.2	22.0

* Bittner, J. J., and Huseby, R. A. Cancer Research, 6: 236. 1946.

fox chow and water. The room temperature was maintained around 80° F.

RESULTS

A. Observations upon the Adrenal

Gross.—The adrenal glands in intact mice of all groups were comparable. Among the older animals the glands were usually firm and pink with the connective tissue capsule occasionally mottled by small circumscribed yellow or deep red areas. Rarely the entire adrenal was yellow.

Comparison of the adrenal glands from the control animals with those glands from the castrated mice brought forth several dissimilarities in the latter. Normal appearing adrenal glands as described in the preceding paragraph were observed among the castrated groups only in mice of the A strain. The adrenal glands from mice of the C3H and hybrid groups had developed definite bulges or nodules. Frequently the glands were yellow and swollen so as to obscure the nodular formations. Differences in size and distortion were noted between the two adrenals of the same animal. The left adrenal was usually the larger. The largest nodules measuring 40 to 50 mm. were found only in the hybrid mice irrespective of the presence or absence of the active milk agent.

Microscopic.—The cortex of the adrenal was principally involved. The distribution of cells throughout the cortex into 3 major zones (zona glomerulosa, zona fasciculata and zona reticularis or X zone) followed the customary pattern in the mouse (11). In the older animals localized regions beneath the capsule were composed of small cells with heavily stained nuclei but with relatively little cytoplasm, an observation also reported by Woolley and Little (Fig. 1), (29).

The adrenal glands in the castrated mice showed considerable involvement of the cortical areas. Two cellular patterns were observed separately or concomitantly within the same adrenal: (a) Extensive areas of small darkly staining cells immediately beneath the capsule arranged in bands from 2 to 12 cells in width, regarded as Type A cells (Figs. 2, 3) and comparable to the smaller localized zones of subcapsular cells present in the adrenal glands of intact animals. (b) Hypertrophied cells in the outer portion of the zona fasciculata. These cells were usually vacuolated, lightly stained with indistinct cell boundaries, oriented in clusters, and corresponded to the Type B (29) or the syncytial cells (12), (Figs. 2, 3).

The adrenal glands were classified into 4 groups as determined by the histological structure:

1. Adrenal glands showing normal cellular arrangements in the cortex constituted one group. Some glands were included, therefore, in which the so-called Type A cell had accumulated beneath the capsule (Fig. 1).

2. The adrenal glands in the second group had cords of the cortical zones disrupted to different extents by cells of Type A and Type B. Type B cells were observed first in the outer cortex where the fasciculata cells appeared swollen (Fig. 2). The glands with more extensive involvement of the zona fasciculata usually showed an increased number of subcapsular Type A cells between the aggregates of Type B cells. Brown degeneration cells were distributed throughout the cortex and in the juxtamedullary zone in variable numbers. Nodules were visible grossly in glands with such cortical rearrangement.

3. Small adenomas were noted in the adrenal glands classified in this group. Large numbers of elongated, spindle-shaped cells of the subcapsular type extended into the main body of the cortex between the groups of Type B cells. The Type B cells themselves were smaller, had less cytoplasm, still stained lightly, were vacuolated but with heavily staining nuclei, many of which were in different stages of mitosis. Cord-like trabeculation of the cells was not observed (Fig. 3).

4. The glands placed in group 4 had the cortical tissue replaced by histologically malignant cells and were considered to be adenocarcinoma (Fig. 4). Resemblance to Type A or B cells was lacking. Cell boundaries were also poorly defined. The undifferentiated cells comprising the tumor were arranged in lobules of different sizes and separated from one another by strands of connective tissue. The blood supply was scant and necrosis was common in the larger tumors.

Amyloid degeneration was observed in the adrenal glands of each of the 4 groups in differing amounts. The medulla was frequently compressed to one side by the overgrowth of cortical tissue but otherwise was not involved. No metastases were observed.

Strain differences among the castrated mice were apparent in the extent of adrenal gland involvement. The glands in mice of the A strain showed no marked pathological lesions. The usual cord-like arrangement of the cortex in the adrenal was modified by subcapsular Type A cells. Adenomas were present in 4 animals. In none of the mice were the normal contours of the organ disturbed (Table II).

More extensive adrenal lesions were noted in the other groups of mice studied. The C3H strain mice,

TABLE II: THE DISTRIBUTION OF ADRENAL CORTICAL LESIONS IN CASTRATED FEMALE MICE OF THE A AND C3H STOCKS AND HYBRIDS BETWEEN THESE STOCKS AS DETERMINED BY HISTOLOGICAL EXAMINATION

Strain	No. of animals	Normal Adrenal glands No.	%	Subcapsular cells and hyperplasia No.	%	Adenoma No.	%	Adenocarcinoma No.	%
A	33	24	72.9	5	15.2	4	12.1	0	00.0
C3H(Z)	33	1	3.3	9	27.3	20	60.6	3	9.0
ZAF_1*	14	5	35.7	2	14.2	4	28.5	3	21.4
AZF_1*	14	2	14.2	1	7.1	6	42.9	5	35.6
Hybrid ZBC*	55	3	5.4	6	10.9	21	38.2	25	45.5
Fostered ZBC†	24	2	8.3	5	20.8	5	20.8	12	50.0

* High tumor hybrids with active milk agent.
† Low tumor hybrids lacking active milk agent.

the high tumor hybrid mice with the milk agent (ZAF_1, AZF_1, hybrid–ZBC) and the low tumor mice without the active milk agent (fostered ZBC) showed considerable cortical involvement. The alterations in the adrenals of the C3H strain mice were less than in the mice of the hybrid groups. Hypertrophied cortical cells were found in the adrenals of all but one mouse of the C3H strain. Sixty per cent of those examined showed adenomatous development and 3 animals (9.0 per cent) showed adenocarcinomas. Brown degeneration in the juxtamedullary zone was conspicuous.

The majority of the hybrid mice with or without the milk agent showed adenomatous or adenocarcinomatous changes of the adrenal cortical cells. Brown degeneration was not localized as among the C3H mice but was scattered throughout the cortex. Although the tumors did not invade surrounding organs, the cortical cells frequently infiltrated through the capsule of the adrenal. Tumors of the lung were present in 9 mice of the high tumor hybrid group and 11 mice of the fostered hybrid group. The pulmonary tumors were characteristic of primary lung tumors of the A strain. The presence or absence of the milk agent had no relationship to the extent of cortical pathology.

B. Observations of Functional Activity of Adrenal Lesions

1. *Vaginal smears.*—Daily examination of the vaginal mucosa of intact animals representing the four main groups of mice being studied indicated: (a) that individual cycles in the animals of each group lasted four to six days, (b) that the resting phase, if present, was short. No attempt was made to determine the time when estrous cycles began in these mice but a high percentage of the animals 3 months old had periodic cornified vaginal smears.

Among the castrated mice the failure of the vaginal orifice in the females of the A strain to open until the animals were 5 or 6 months old made observations previous to this period impossible. At no time during subsequent examinations were smears of the estrous type obtained (Table III).

Animals of the other groups showed cornification as early as 3 months following castration. The vaginal canal was open at the time of castration or shortly thereafter. A series of daily observations of

TABLE III: PERCENTAGE OF INTACT AND CASTRATED FEMALE MICE OF THE A AND C3H STOCKS AND HYBRIDS BETWEEN THE TWO STOCKS OBSERVED WITH ESTROUS VAGINAL SMEARS (4 TO 18 MONTHS)

Strain	Intact mice No.	%	Castrated mice No.	%
A	15	93.3	54	00.0
C3H(Z)	8	75.0	62	74.0
High tumor hybrids*	14	92.0	69	80.0
Low tumor hybrids†	—	—	56	67.8

* Hybrids with active milk agent.
† Hybrids lacking active milk agent.

the vaginal smears revealed, (a) that cycles, when present, were irregular, (b) that most animals gave smears showing both cornified cells and leukocytes, and (c) that a few animals were in diestrus throughout the 2 week period of examination.

2. *Uterus.*—A second end organ stimulated by steroid hormones is the uterus. The uteri increased in size and in blood supply in intact mice of all groups. Depending upon the time during the estrous cycle at which the tissue was taken, evidence of both estrogenic (tall columnar epithelium, intercellular edema, spindle-shaped stromal nuclei) and progestational activity (cuboid epithelial cells, enlarged granular stromal nuclei) was present (Fig. 5) (1, 18).

All castrated mice of the A strain, regardless of age, had small and thread-like uteri. In contrast, mice of the C3H strain and of the hybrid groups had large well vascularized uteri.

Microscopically the differences between the mucosa of the uterus in mice of the A strain and that of animals in the other groups were apparent. In addition to smallness of the uterine diameters of mice of the A strain the glandular development, blood supply and muscle were scant (Fig. 6). The uteri in mice of the C3H strain and hybrid stocks

exhibited indications of hormonal activity; namely, cystic hyperplastic endometrium, tall columnar epithelia, numerous mitotic figures in the surface glands and in the deeper mucosa, edema, stromal nuclear changes varying from small spindle-shaped cells to enlarged rounded types with vesicular nuclei, leukocytes and an increase in fibrous connective tissue. Different combinations of these indicators suggested estrogenic, androgenic and progesterone influences (Fig. 7).

3. *Mammary glands.*—A third indicator of hormonal activity was the structure of the mammary glands (1, 16). The mammary architecture in the intact mice of the A strain was primarily ductular (Fig. 8). Greater alveolar development was apparent in mice of the other groups. Hyperplastic nodules and mammary tumors were found only in mice of the C3H strain and of the high tumor hybrid group (Fig. 9). The mammary glands of the fostered mice lacked hyperplastic nodules (Fig. 10).

The glands of the castrated mice had unusually long duct systems. Side branching was slight and the distances between lateral branches were variable. The type and degree of development showed considerable variation even within mice of the same strain. The castrated mice of the A strain showed greatest deviation from the usual appearance. Frequently 1 or 2 of the glands were of the rudimentary male type while the remainder of those studied were more extensively developed. As a rule the glands consisted of a main duct with a few side branches and no hyperplastic nodules (Fig. 11). Two mammary tumors developed (Table IV).

Castrated mice of the C3H strain and mice of the high-tumor hybrid stocks had mammary glands similar to those in mature virgin mice of that stock. The lateral branching and alveolar development were somewhat less than that in the intact animal. The ducts and alveoli in parts of the gland were distended with secretion. Hyperplastic nodules and mammary tumors were present (Fig. 12, Table IV).

In the castrated mice lacking the milk agent, the mammary ducts were similar to those of mice in the C3H strain and high tumor hybrid stocks with extended smaller ducts and irregular alveolar development. No hyperplastic nodules and no mammary tumors were present (Fig. 13, Table IV).

The average age at which the tumors appeared in mice of the castrated series, with the exception of the A strain, was somewhat later than in the groups of virgin mice. There was no consistent correlation in the age of the animals dying noncancerous between the castrated and virgin groups (Tables I, IV). Although the presence of Type A or B cells in the adrenals of the castrated mice and the postulated hormonal activity were related to the extent of mammary tree development, the adrenal lesions influenced mammary tumor development only in the presence of an active milk agent. The few cellular changes in the cortex of the adrenal glands in mice of the A strain are reflected in the immature male-like structure of the mammary gland whereas more extensive cortical changes in mice in the other groups coincide with the greater development of the mammary gland and mammary tumors in the susceptible stocks.

DISCUSSION

Removal of the ovaries from mice of certain strains was followed by gross and microscopic changes in the uterus, vagina and mammary glands showing stimulation by estrogenic, androgenic or progesterone-like substances. The adrenal cortical hyperplasia and carcinomas which were observed in these animals were suggested as the source of the stimulating hormones (13, 14, 27, 30). When the ovarian hormones were replaced by administration of diethylstilbesterol (31) or by certain androgenic steroids (26), the hyperplastic cellular changes in the adrenal cortex were prevented, and the adrenal glands were established as the source of the steroids involved.

The nature of the hormones being released by the hyperplastic cells in the adrenal glands of the cas-

TABLE IV: SUMMARY OF DATA SHOWING THE INCIDENCE AND TIME OF OCCURRENCE OF MAMMARY TUMORS IN CASTRATED FEMALE MICE OF THE A AND C3H STOCKS AND HYBRIDS BETWEEN THE TWO STOCKS WITH THE PERCENTAGE OF ADRENAL TUMORS IN EACH GROUP

Strain	No. of animals	No. of mammary tumors No.	%	Ave. ca. age, months	Ave. non-ca. age, months	Adrenal tumors,* %
A	46	2	4.3	6.7	16.3	4.0
C3H(Z)	53	29	54.7	14.2	16.0	69.0
ZAF_1†	14	10	71.5	20.9	19.5	50.0
AZF_1†	35	19	54.4	19.3	20.0	77.0
Hybrid ZBC†	69	36	52.0	16.4	17.5	83.0
Fostered ZBC‡	55	0	—	—	20.0	70.0

* Tumors include adenomas and adenocarcinomas.
† High tumor hybrids with active milk agent.
‡ Low tumor hybrids lacking active milk agent.

trated mice was ill-defined. Present evidence has suggested that the secretions from the pituitary gland influenced the liberation of hormone from the adrenal cortex in the castrated animals. The administration of gonadotrophic hormones has been demonstrated to discharge the hormones from adrenals in castrated animals (14). Adrenotrophic hormone has not been effective in the doses tried (20). Caloric restriction has lowered the hormonal content and activity of the hyperplastic adrenal cortical cells (19). Because the type of cellular change observed in the adrenals of the castrated mice of the several strains was essentially similar but the extent of the lesion varied considerably, a differential pituitary action upon the adrenal cortex was probable. A differential response to pituitary stimulation by the adrenal may also not be excluded.

The variability in mammary gland development, the difference in uterine development and the superimposition of leukocytes upon cornified epithelial cells in the vaginal smears suggested that, (a) several types of steroid hormones were being released from the adrenal cortex in the castrated mice, (b) the hormones were being released in varying quantities, (c) the metabolism of the hormones present was being handled differentially, or (d) the end organ response to the several steroids was peculiar to the individual strain and animal. The quality and quantity of the secretion in the present experiment could be determined only insofar as was observed that castrated mice of the A strain with scant adrenal cortical pathologic change showed little evidence of hormonal activity when contrasted with the extensive lesions in the C3H and hybrid groups in which there was considerable evidence of hormonal activity.

The selection of two strains (A and C3H) highly inbred for the production of mammary tumors when maintained as breeding stock, permitted a study of the genetic factors influencing the formation of adrenal tumors as compared to the effectiveness of these factors in the formation of mammary tumors.

All the strains studied in the present experiment have an inherited susceptibility for mammary tumors. An active milk influence is present in each of the groups except the fostered hybrid stocks. All strains but the A strain have the inherited hormonal influence (Table V).

Mice of the A strain, lacking the inherited hormonal influence, failed to show the extreme adrenal cortical changes following castration which were present in the majority of the animals in the other groups. No ovariectomized mice of the A strain had estrous cycles. There was little or no uterine growth. The mammary gland development was slight. No precancerous nodules and but 2 mammary tumors were present.

The animals in the C3H strain and in the high tumor hybrid stocks in which all 3 factors for mammary tumor development were present, had extensive adrenal cortical pathologic change following castration. Vaginal cornification was observed. Mucosal changes indicative of hormonal stimulation were present in the uterus. Mammary glands were comparable to those in the intact animals and contained hyperplastic nodules and tumors. The low tumor hybrids, derived from mice lacking the milk agent, showed changes in the adrenal, uterus, and vagina similar to those observed in the C3H and high tumor hybrid stocks. No precancerous lesions in the mammary glands and no mammary tumors were observed.

Because only slight changes were found in the adrenal glands of mice in the A strain following castration, and because mice of this strain are also believed to lack the inherited hormonal influence, the extensive changes in the C3H and hybrid groups of mice, in which the inherited hormonal influence is present, suggest that the inherited hormonal influence functions genetically and may be considered as a mechanism influencing the cellular changes in the adrenal cortex following castration.

SUMMARY

1. The three factors known to influence the growth of mammary tumors in inbred strains of mice have been studied in relationship to the formation of adrenal tumors following castration.

TABLE V: CORRELATION OF OBSERVATIONS UPON CASTRATED MICE OF THE A AND C3H STOCKS AND HYBRIDS BETWEEN THE TWO STOCKS WITH AND WITHOUT THE ACTIVE MILK AGENT

	A strain	C3H strain	High tumor hybrids	Low tumor Hybrids
Genetic susceptibility to Mammary Cancer	Present	Present	Present	Present
Milk influence	"	"	"	Absent
Inherited hormonal influence	Absent	"	"	Present
Adrenal cortical lesions	27.1%	96.7%	91.7%	91.7%
Uterine stimulation	Absent	Present	Present	Present
Estrous activity	"	"	"	"
Mammary gland nodules	"	"	"	Absent
Mammary tumor incidence	4.3%	54.7%	55.0%	00.0%

2. One of these factors, modifying hormonal activity in breeding and virgin mice, and designated as the inherited hormonal influence was observed to be in castrated mice of the C3H stock and to be transmitted as a dominant to the hybrids between the A and C3H stock but to be lacking in the castrated mice of the A strain.

3. Adrenal cortical hyperplasia and tumors occurred in those castrated groups with the inherited hormonal factors (C3H, high and low tumor hybrid stocks).

4. The hyperplastic adrenal glands were suggested as the source of compounds similar to those released by the ovary in the intact animal. These hormones are believed to be responsible for the evidence of hormonal activity in the vagina, uterus and mammary glands.

5. Mammary tumors were present in the castrated mice with the inherited hormonal influence provided an active milk agent was present (C3H, high tumor hybrids).

6. The castrated mice of the A strain, lacking the inherited hormonal influence even in the presence of the genetic susceptibility to mammary tumor formation and an active milk agent, had slight adrenal lesions and little or no indication of hormonal stimulation of the uterus, vagina or mammary glands.

7. The action of the inherited hormonal influence is probably mediated at least in part through the hypophysis.

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DESCRIPTION OF FIGURES 1 TO 7

Fig. 1.—Adrenal cortex from mouse of intact group showing slight subcapsular cell proliferation with penetration of these cells into zona fasciculata. Mag. × 300.

Fig. 2.—Adrenal cortex from castrated mouse showing subcapsular cell proliferation, hypertrophy and hyperplasia of cells in zona fasciculata. Mag. × 175.

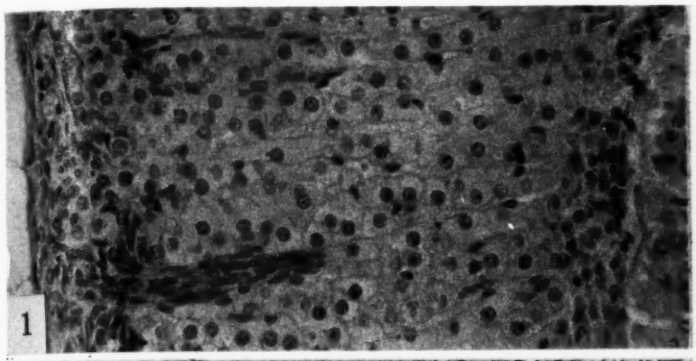
Fig. 3.—Cross section of adrenal gland from castrated mouse showing dense areas of subcapsular cell proliferation associated with normal portions of cortex, and showing hyperplastic and adenomatous nodules involving remainder of cortex. Mag. × 6.

Fig. 4.—Area of undifferentiated cells undergoing active mitoses from histologically malignant adrenal cortical tumor in castrated mouse. Mag. × 175.

Fig. 5.—Cross section of the uterus from intact mouse. Mag. × 85.

Fig. 6.—Cross section of uterus from castrated mouse of the A strain. Lack of glandular development, compactness of lamina propria and scant muscular elements are characteristic. Mag. × 85.

Fig. 7.—A cross section of the uterus from a castrated hybrid mouse. The cystic endometrium, changes in stromal nuclei and well developed muscle tissue are apparent. Mag. × 85.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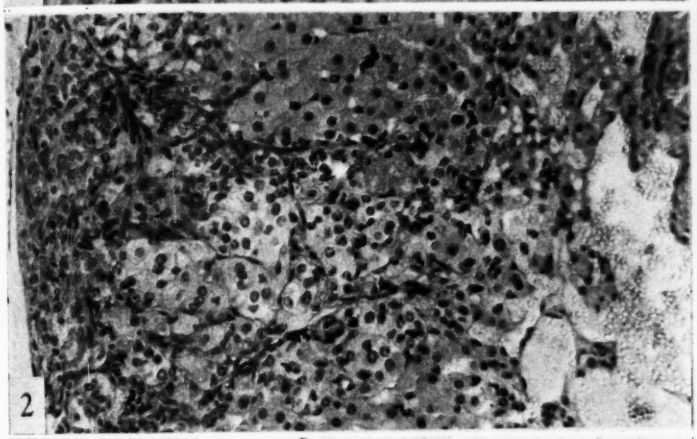
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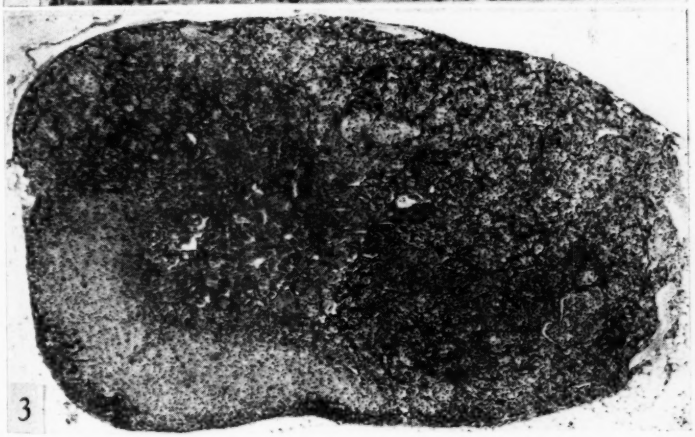
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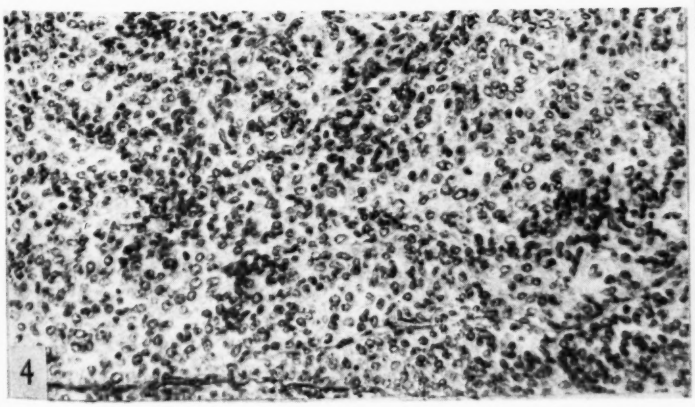
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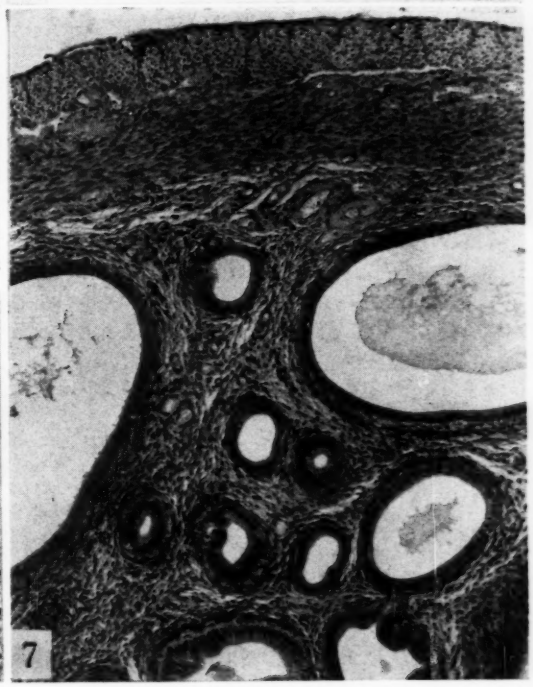
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DESCRIPTION OF FIGURES 8 TO 13

FIG. 8.—Whole mount of mammary gland from intact virgin mouse of the A strain. The main duct system is complex but alveolar development is scant. Mag. × 35.

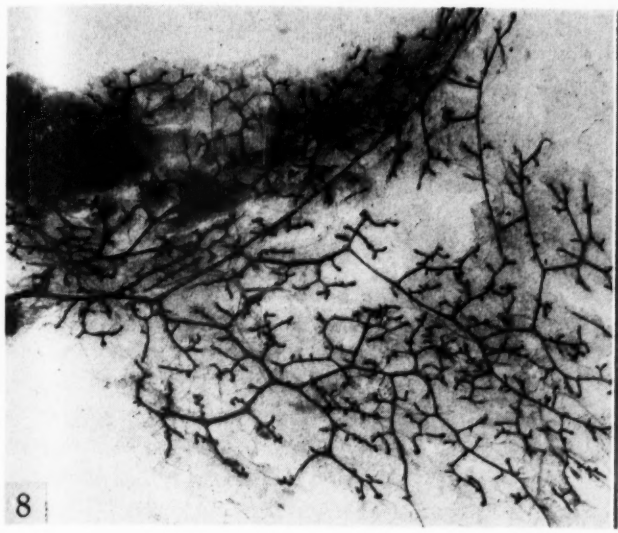
FIG. 9.—Whole mount of mammary gland from intact virgin hybrid mouse. Hyperplastic nodules and some alveolar development are apparent. Mag. × 35.

FIG. 10.—Whole mount of intact virgin mouse lacking the milk agent. No hyperplastic nodules are present. Mag. × 35.

FIG. 11.—Whole mount of mammary glands from castrated mouse of the A strain. The glands are of rudimentary male type. Mag. × 35.

FIG. 12.—Whole mount of mammary gland from castrated hybrid mouse. Hyperplastic nodules are present as in normal virgin mice but there is less alveolar development. Mag. × 35.

FIG. 13.—Whole mount of mammary gland from castrated hybrid mouse lacking the milk agent. Extensive but irregular development of duct system is present. Mag. × 35.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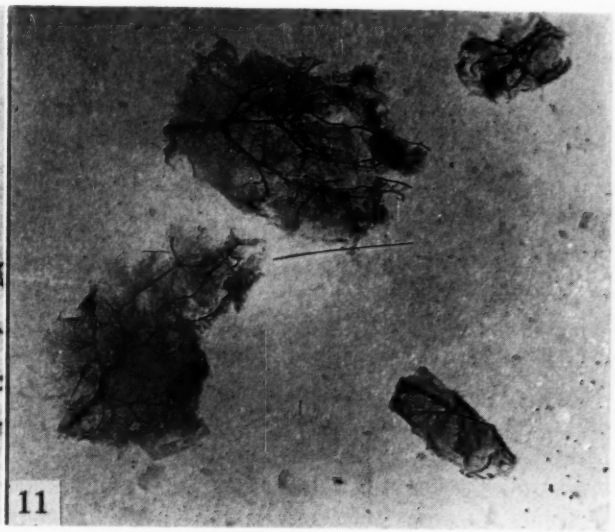
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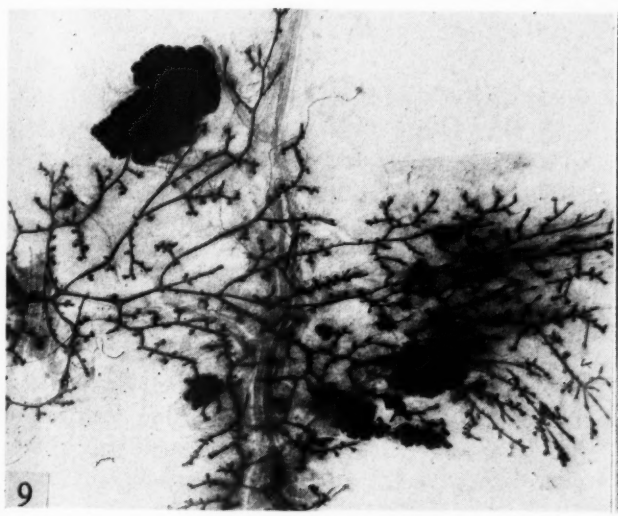
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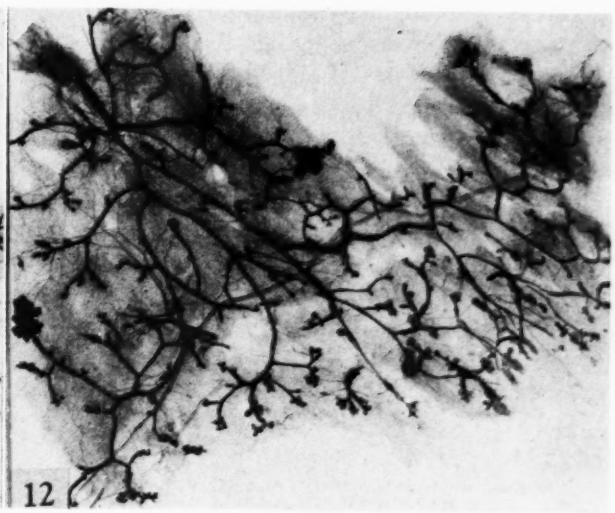
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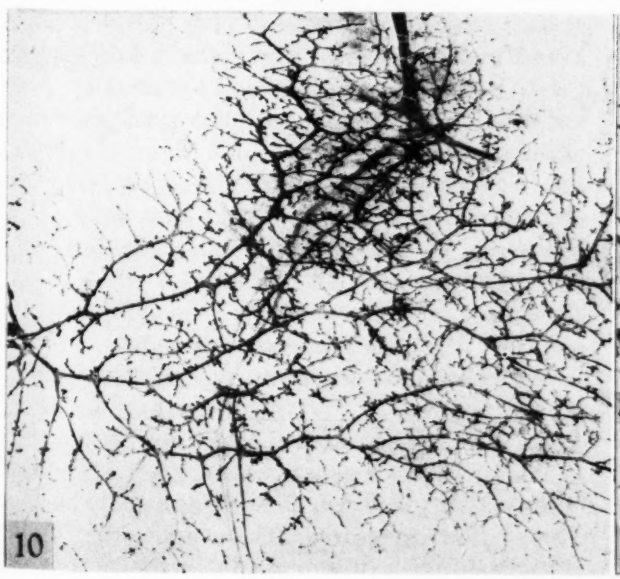
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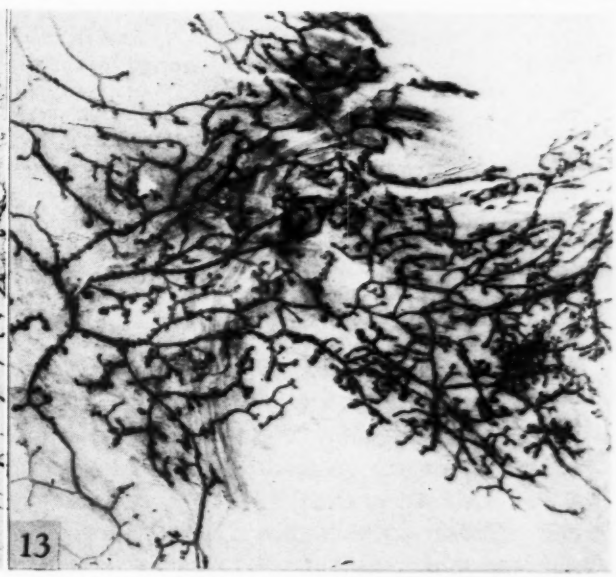
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The Diphenylamine Reaction of Human Serum

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In the course of investigating differences between the sera of patients suffering from malignant tumors and apparently well persons, it was found that the addition of the diphenylamine reagent (1, 2) to certain fractions of human serum resulted in the production of a purple color whose intensity varied in different individuals. We wish to make a preliminary report on our observations concerning this phenomenon.

TECHNIC

To 2 ml. serum in a Pyrex tube (25 × 150 mm.) are added 4 ml. 20 per cent trichloracetic acid. The tube is gently shaken and centrifuged for 15 minutes at 2,000 R.P.M. The supernatant, a fluid which is negative for diphenylamine reaction, is poured off.

To the precipitate are added 5 ml. of 5 per cent trichloracetic acid, and the precipitate is well mixed with the acid by means of a thin glass rod which is well shaken in the mixture so that nothing adheres to it when removed. The tube is covered with a glass stopper and heated for 15 minutes in a vigorously boiling water bath. It is then removed from the bath and cooled by immersing in cold water. The tube is then centrifuged for 15 minutes at 2,000 R.P.M. and, the clear colorless supernatant fluid is drawn off and recentrifuged for another 15 minutes.

In a Pyrex tube (22 × 174 mm.) are placed 3 ml. of this final clear colorless solution and in another, which is the blank, 3 ml. of 5 per cent trichloracetic acid; 6 ml. of the diphenylamine reagent (100 ml. glacial acetic acid plus 2.75 ml. concentrated sulphuric acid plus 1 gm. diphenylamine) are added to each of the 2 tubes and both tubes are covered with glass stoppers and are heated for exactly 10 minutes in a vigorously boiling water bath and then simultaneously removed from the bath and cooled in running cold water.

The purple color that is developed is read in the Evelyn photocolorimeter against its blank, which is the tube containing the 5 per cent trichloracetic acid and the diphenylamine reagent, using a 660 millimicrons filter. The intensity of purple color is recorded as density values ($L = 2 - \text{Log. } G$), where G is the galvanometer reading.

OBSERVATIONS

The substance or substances in the fractions of human serum which give the purple color with diphenylamine are present in all individuals studied. The amount however, varied, being greater in patients with malignancies than in individuals who were apparently well (Table I). Fifty-six out of a total of 62 patients with malignancies had L—values for the diphenylamine reaction exceeding 0.45. The types of malignancies and the organs involved are shown in Table II. In the main, the malignancies studied were well advanced, but in seven individuals the malignancies were well localized and had produced no systemic or wasting effect on the patients. The L-values, moreover, in these seven cases were well beyond 0.45.

Fifty-three individuals who were apparently well were also studied. Mainly, these people were active hospital personnel and their ages varied from 20 to 65 years. In all instances the L values of the diphenylamine reaction in this group fell below 0.45 (Fig. 1, Table I).

TABLE I: THE DIPHENYLAMINE REACTION OF NORMAL AND PATHOLOGICAL HUMAN SERA

	No.	L value	Mean L value
Normal sera	53	0.220–0.450	0.337
Malignant tumors	56	0.475–0.969	0.525
Benign tumors	3	0.237–0.362	0.320
Pregnancy	6	0.310–0.432	0.382
Postpartum	9	0.469–0.602	0.507
Non-neoplastic disease	6	0.237–0.444	0.347
Inflammations	5	0.502–0.824	0.623
Pulmonary tuberculosis	2	0.509–0.523	0.516
Acute rheumatic fever	2	0.585–0.602	0.594

We have also examined 2 patients with benign tumors of the breast and 1 with leiomyoma of the stomach, and these had L values below 0.45. Others with non-neoplastic diseases, among them 2 with gastric ulcer, 2 with cirrhosis of the liver, one with toxic goiter, and 2 with diabetis mellitus, all exhibited L values of the diphenylamine reaction in the fractionated serum below 0.45. However, 2 patients with pulmonary tuberculosis, 1 with chronic

nonspecific empyema, 1 with cellulitis of the chest wall, 1 with chronic osteomyelitis of the femur, 2 with rheumatic fever, and 1 with sub-acute bacterial endocarditis and 1 with bronchiectasis had *L* values above 0.45.

With the exception of one case,[1] pregnant (7) patients at varying stages of gestation had *L* values below 0.45. However, during early postpartum *L* values exceeded 0.45. But as only 10 postpartum patients were studied, the number was not sufficiently great nor the follow-up long enough to warrant further comment at this time.

TABLE II: THE TYPES OF MALIGNANCIES STUDIED

Type of malignancy	Site	No. of cases
Carcinoma	Stomach	14
"	Anus	3
"	Esophagus	2
"	Breast	5
"	Parotid	1
"	Colon	3
"	Maxillary antrum	1
"	Lip	2
"	Buccal mucosa	1
"	Lung	3
"	Pancreas	1
"	Gall bladder	1
"	Palate	1
"	Metastatic in cervical lymph node, primary?	1
"	Cervix uteri	3
"	Penis	1
"	Face	5
"	Alveolar ridge	2
"	Urinary bladder	1
"	Prostate	3
"	Rectum	1
"	Ovary	1
Myosarcoma	Thigh	1
Spindle cell sarcoma	Foot	1
Hodgkin's disease	Generalized	3
Leukemia		1
	Total	62

DISCUSSION

Our first attempts at testing whole serum with the diphenylamine reagent were unsuccessful because the colored solutions obtained even after filtration through a Finster filter were cloudy and thus did not lend themselves to true readings of color intensity with the Evelyn photocolorimeter. By precipitation with 20 per cent trichloracetic acid, then boiling the precipitate further with 5 per cent trichloracetic acid, the colored solutions obtained after the addition of the diphenylamine reagent were clear and could be compared accurately with controls in the photocolorimeter. We believe this is an important step in the preparation of our specimens. The nature of the substance or substances which, with the diphenylamine reagent, produce the purple color is not yet known, but probably it is carbohydrate in nature and is extracted by the trichloracetic acid hydrolysis of the protein precipitate of the serum (1, 2). The important observation, we believe, is that the intensity of the purple color is greater in patients with malignancies than in individuals who are apparently well. The differences in *L* values between the malignant and "normal" sera are statistically significant.[2] As noted above, most of the malignancies studied were well advanced, but even in those cases where the neoplastic process was well localized and without any marked systemic effect upon the individual, the *L* values of the fractionated serum were higher than the "normal" range. The few cases of benign tumors and other non-neoplastic diseases such as peptic ulcer, cirrhosis of the liver, toxic goiter, and diabetes mellitus which were studied all had lower *L* values than patients with malignant tumors.

That this increased intensity of purple color subsequent to the addition of diphenylamine reagent is not specific for malignancy is obvious from the following facts: (a) a few cases of malignancies had *L* values in the "normal" range. (b) postpartum patients and patients with tuberculosis, empyema, cellulitis, osteomyelitis, rheumatic fever and subacute bacterial endocarditis all gave *L* values equal to those obtained in patients with malignancies. Further work is being carried out by us to determine the exact nature of the substance or substances responsible for the purple color after the addition of diphenylamine and to determine the value of the diphenylamine reaction as a diagnostic test for the presence of malignancy. To date, in a series of 82 "unknowns" there were 27 patients with malignancies, and of this group 23 were diagnosed correctly and 4 were missed. In the remaining 54 there were 45 patients who were perfectly well and all were found to have *L* values below 0.45. The remaining 10 had either an acute or chronic inflammatory process and these, with the exception of one with chronic ulcerative colitis, had *L* values above 0.45.

[1] This patient also had subacute bacterial endocarditis.

[2] The "chi-square" criterion was applied to ascertain the probability that this type of marked divergence in the two frequency distributions might be expected solely through chance. χ^2 proved to be 95, and from this the probability is so small as to be beyond the tables available to us, which end at one in a million. It may therefore be considered proven beyond any reasonable doubt that normal persons and those with malignant tumors differ very significantly in their *L*-value distributions.

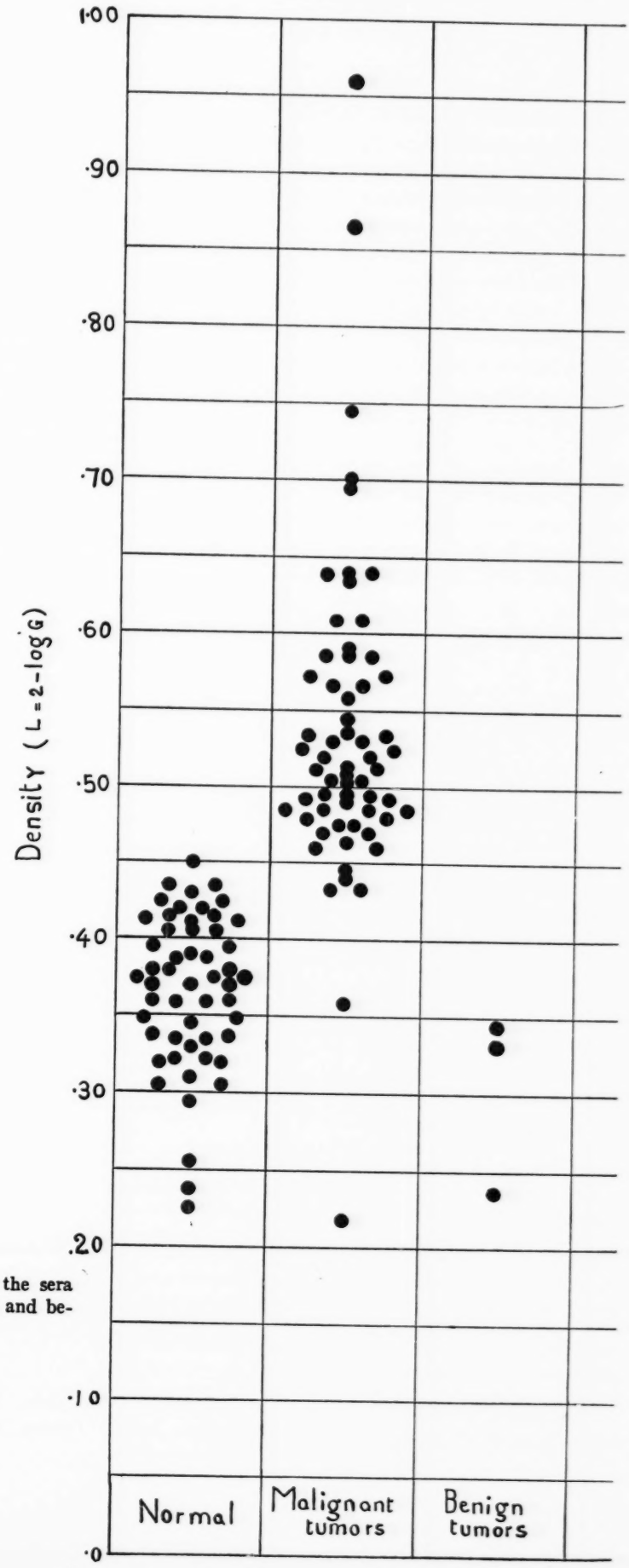

FIG. 1.—Density of diphenylamine reaction in the sera of normal individuals and patients with malignant and benign tumors.

CONCLUSIONS

1. The addition of the diphenylamine reagent to certain fractions of human serum result in a purple color, the intensity of which is greater in patients with malignancy than in individuals who are apparently well.

2. In a small number of patients with benign tumors, gastric ulceration, cirrhosis of the liver, toxic goiter, diabetes mellitus and pregnancy the *L* values after the addition of the diphenylamine reagent were lower than in cases of malignancy.

3. The intensity of the purple color is increased beyond "normal" (0.45) range in postpartum patients and in those with tuberculosis, empyema, cellulitis, rheumatic fever and subacute bacterial endocarditis.

4. The nature of the substance or substances responsible for the purple color is unknown. Work is in progress to determine their nature.

5. The value of the diphenylamine reaction in human serum as a diagnostic test for cancer is being investigated.

ACKNOWLEDGMENT

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Tumors of Fishes, Amphibians, and Reptiles*

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The study of tumors occurring spontaneously in animals and the experimental study of neoplasia are complementary. Such studies in the past have dealt largely with tumors in the higher vertebrates, mammals and birds; much less attention has been paid to the lower vertebrates, fishes, amphibians, and reptiles. But neoplastic processes are ubiquitous among vertebrates, and comparison has shown that tumors in cold-blooded animals are essentially identical in regard to their structure and behavior with the corresponding tumors of warm-blooded animals. Since no comprehensive account of spontaneous tumors of the former group exists, it was thought advisable to assemble the available literature as a guide to source material and as an aid to future investigations. This has been done in the present paper. In a complementary paper are reviewed the lines of experimental investigation which have been pursued (123a).

I. TUMORS OF FISHES

Fishes† are the most numerous class of vertebrates (73). Because of their economic importance, many species are caught in vast quantities. During the marketing and preparation of the catch for food, any existing tumors are apt to be noticed. For these reasons information about the neoplastic diseases of fishes is more adequate than for amphibians and reptiles. All the major varieties of tumors that occur in mammals, including man, and in birds have been recorded in fishes. These tumors will now be taken up in the following order: Tumors of epithelial tissue, tumors of mesenchymal tissues, pigment cell tumors, tumors of nervous tissues and hamartomas.

TUMORS OF EPITHELIAL TISSUES

In this group are included: papilloma, adenoma, tumors of dental origin, epithelioma, adenocarcinoma, "tumors" of the thyroid.

PAPILLOMA

The most common benign epithelial tumors of fish hitherto recorded are papillomas, which have been reported in at least 16 different species (Table 1). The tissue of origin in all is surface epiderm;‡ papillomas of the viscera have not as yet been observed. The tumors vary in appearance; some are finely lobulated, rather flat elevations; others, small round warty growths, and still others, large cauliflower-like masses. They are usually solitary, but multiple tumors are not rare. Histologically the tumors have typical papillomatous arrangements and show no tendency to invade the subjacent tissues.

Of particular interest are the more common tumors, some of which are perhaps transmissible. Among them are the small, multiple, wart-like papillomas described by Fiebiger (47). They developed in a group of climbing perch (*Anabas scandens*) which had been kept in the same aquarium for two years. The tumors were scattered over various parts of the body and ranged in size from that of a poppy-seed to a pea. Microscopic examination revealed marked hyperplasia of the epidermis and papillary proliferation of the connective tissue of the corium; the latter was heavily infiltrated with leukocytes. Fiebiger called attention to the resemblance of these tumors to the infectious warts of mammals.

Another common tumor was described by Keysselitz (97) in barbels (*Barbus fluviatialis*) of the Mosel River. The growths were pea-sized, usually single, round or oval papillary masses located on the lips; rarely they extended to the adjacent skin. Histologically, closely packed epithelial masses were supported by coarse fibrous trabeculae which extended upward from the corium and were often infiltrated with leukocytes. Mitoses were absent. The nuclei of many epithelial cells contained one or more

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† In this review nomenclature for species follows current usage; where necessary the older names used in the reports have been replaced.

‡ The epidermis of fishes is composed of stratified squamous epithelium in which typical keratinization as seen in man does not occur. Large numbers of mucous cells are present at all levels in the epidermis; they ultimately reach the free surface where they empty their contents. Characteristic of the epidermis of cyclostomes and teleost fishes are the very large "clavate" cells. These elongated club-shaped cells pass vertically through several layers of the squamous epithelial cells. The bulk of the cell body is composed of a homogeneous eosinophilic substance which probably is a secretion of the cell rather than living cytoplasm. In the center of the clavate cell is a small nucleus surrounded by a halo of clear cytoplasm. Detailed descriptions of the morphology of the skin of fishes are found in the articles by Kann (94) and Graupner and Fischer (64).

TABLE 1:* PAPILLOMA

Species	Site	Author
Gudgeon *Gobio* (several species) (4)	Skin	Schroeders, 1908
Barbel *Barbus fluviatialis* (several)	Lips	Keysselitz, 1908
Climbing Perch *Anabas scandens* (several)	Body surface	Fiebiger, 1909a
Halibut *Hippoglossus hippoglossus*	Snout	Johnstone, 1912
Stint *Osmerus eperlanus* (37)	Lips, buccal cavity, fins	Breslauer, 1916
Gudgeon *Gobio nigronatatus* (2)	Skin	Anitschkov & Pavlovsky, 1923
Gudgeon *Gobio blenniodes*	Skin	Anitschkov & Pavlovsky, 1923
Plaice *Pleuronectes platessa*	Body surface	Johnstone, 1925
Plaice *Limanda limanda* (3)	Body surface	Johnstone, 1925
Goldfish *Carassius auratus* (2)	Fins	Sagawa, 1925
Crucian Carp *Carassius carassius*	Skin of operculum	Takahashi, 1929
Sole *Solea solea* (2)	Skin of trunk	Thomas, 1930
Eel *Anguilla anguilla* (3)	Lower lip	Thomas & Oxner, 1930
Brown trout *Salmo trutta*	Skin of back	Thomas, 1932e
Electric eel *Electrophorus electricus*	Skin	Coates, Cox & Smith, 1938
Slippery Dick *Iridio bivittata* (30)	Skin	Lucké, 1938
Sucker *Catostomus commersonnii*	Skin near tail	Lucké & Schlumberger (not published)

* In this and the succeeding tables, the number of tumor-bearing animals, when in excess of one, follows the name of the species.

inclusions which were surrounded by an achromatic halo, and could be readily distinguished from nucleoli. In what appeared to be their earlier stages they were dense and compact; later they became fluffy and less distinct. The author compared them with the inclusions of vaccinia and other virus diseases.

In 37 specimens of the stint *Osmerus eperlanus* Breslauer (16) found tumors that likewise occurred mainly on the lips, less commonly on the mucosa of the oral cavity or on the fins. The fish were taken from the brackish water of an inlet of the Baltic sea. Upper and lower lips were affected equally; frequently the tumors were in apposition. Some were cauliflower-like growths, others were flat, varying in size from small nodules to masses as large as the head of the affected fish. Histologically all had a papillomatous structure. Usually they showed no tendency to invade, but occasionally the arrangement of the cells suggested early malignancy. Cytoplasmic inclusions were sometimes, but not regularly, encountered.

It may be pertinent here to discuss the tumors reported by Schroeders (179) and by Anitschkov and Pavlovsky (3) in several species of gudgeon *Gobio*, a small marine fish. The descriptions of the latter authors particularly exemplify the difficulty of classifying certain skin tumors of fishes; 3 tumors which they regarded as distinct types may represent merely different stages of the same neoplastic process. In their first case there were numerous small nodules and flattened elevations, the larger and more fully developed ones distinctly papillomatous, with their bases and the subjacent corium the site of a pronounced inflammatory reaction; the authors likened these growths to the chronic inflammatory papillary hyperplasia of the skin and mucous membranes in man. In the second case a large rather flat solitary growth was found on the dorsum of the fish, *Gobio nigronatatus*. Histologically this had the character of a benign papilloma and was not associated with an inflammatory process. In the third case a relatively massive, broadly sessile, cauliflower-like tumor, 2 x 1 x 1 cm., occupied the dorsum of a small *Gobio blenniodes*. It differed from the growth of the second case in the markedly irregular hyperplasia of its epithelial covering and in the delicacy of the supporting connective tissue trabeculae. The tumor did not infiltrate the subjacent tissues, and although the authors regarded it as a papillary carcinoma, the evidence for malignancy is inconclusive. The tumors reported by Schroeders (179) are in the same genus of fish, *Gobio*, and have the character of benign papillomas. In the absence of information regarding the further development and fate of these tumors, it is impossible to be certain of their true nature.

Mention must here be made of a non-neoplastic disease of fish, which has sometimes been confused with true tumor, the so-called pox-disease. It is particularly common in carp and may attain epidemic proportions. The disease has no relation to the various kinds of pox of man. Numerous milk-white, flattened elevations are present on the epidermis of the affected fish; so that it appears as if splashed with melted paraffin which has solidified. All parts of the body, including eyes and fins, may be affected. Microscopically the lesions present a simple hyperplasia of the epiderm; the corium usually remains unchanged and sends no papillae into the epithelial mass; however, a papillary structure is occasionally attained by the larger and more persistent growths. On histologic grounds no sharp dis-

tinction can be drawn between these lesions and true neoplasms, but clinically the lesions of pox, unlike true tumors, usually regress and disappear entirely. A detailed and well illustrated account of this disease is given by Plehn (162).

To the cases of papilloma reported in the literature we are able to add new examples from two other species. The first concerns 2 large tumors which affected a common sucker *Catostomus commersonnii* caught in a Wisconsin Lake. The fish was 8 years old (as determined from examination of its scales), its total length was 31 cm., the sex was uncertain. The tumors were located on the right side; the larger measured 6.1 x 5.5 x 4.0 cm. and involved almost the entire lower half of the tail fin, the smaller tumor, which measured 20 mm. in diameter, was located on the tail, immediately in front of the caudal fin (Fig. 1). Both neoplasms were firm, hemispherical masses which had the typical lobulated appearance of papilloma (Fig. 2). Histologically, a delicate arborescent stroma supported great masses of epithelial cells (Fig. 3). Tall columnar cells formed the layers adjacent to the connective tissue stroma; toward the center of the epithelial masses the cells become polyhedral and in places suggest "pearl" formation (Fig. 4). The nuclei were conspicuous, and usually contained one or two prominent nucleoli. Among some of the epithelial masses were groups of large clavate cells (see footnote ‡); mucous cells were not found. The tumors were sharply circumscribed, and nowhere extended into the subjacent tissue; there was no inflammatory reaction.

Another kind of papillomatous growth occurred in a small fish, the slippery dick *Iridio bivittata,* which inhabits tropical and semitropical waters (116). Thirty tumor-bearing specimens were found among approximately 6,000 of these fish examined at the Dry Tortugas, Florida. The tumors had the appearance of flattened nodular elevations of the skin; they were grayish in color and soft in consistency, and often they attained large size (Fig. 5), were most usual on the lateral surfaces and commonly destroyed the fins. Histologically, delicate fibrous projections of the corium were covered with many layers of epithelial cells (Fig. 6). The growths frequently extended into the corium, but infiltration of the deeper tissues was not observed. The cells of most tumors were much larger than the cells of the normal epiderm; the cytoplasm was faintly granular; the nuclei large and round or oval (Fig. 7). The nature of this neoplastic disease is uncertain; whether these growths are true neoplasms or merely exaggerated non-neoplastic epidermal proliferations is yet to be determined. In some respects they seem to be related to the tumors of gudgeons discussed above. Since fish affected with these tumors can readily be kept in indoor sea water aquaria, they should prove excellent material for experimental investigation.

Abstracts of other reported cases of papilloma

SCHROEDERS, 1908: In several species of gudgeon *Gobio* the author observed "fibro-epitheliomata." In some fish the tumors were solitary, in others multiple. In all instances they were sharply circumscribed, papillomatous, and yellow-white in color.

JOHNSTONE, 1912: An irregular cauliflower-like growth was observed on the snout of a halibut *Hippoglossus hippoglossus*, weighing 120 pounds. On section there was marked proliferation of the corium, the overlying epithelium was intact with little evidence of hyperplasia.

JOHNSTONE, 1925: About 1 dozen whitish warts, averaging 5 mm. in diameter, were scattered over the unpigmented side of a plaice *Pleuronectes platessa.* The growths were found to be impregnated with crystals of magnesium phosphate. The corium was thickened and showed evidence of connective tissue proliferation; the overlying epithelium was thin.

JOHNSTONE, 1925: Flat cutaneous warts were found on three plaice, *Limanda limanda* caught in the North Sea. One fish had a single tumor, 5 cm. in diameter, near the eye; another had multiple warts scattered over the body, and the third had 2 growths, one supraorbital and another on the dorsal fin. All of the tumors were papillomas.

SAGAWA, 1925: In each of 2 goldfish *Carassius auratus* there was a wart-like tumor the size of a pea on each of the pectoral fins. The hyperplastic epithelium was arranged in papillary folds and in some areas appeared keratinized. Clavate and mucous cells were absent in the tumor, though they were plentiful in the normal skin.

TAKAHASHI, 1929: In a crucian carp *Carassius carassius* a tumor was located near the posterior margin of the left operculum. It was a sharply circumscribed grayish-white growth, 3 x 2 x 0.5 cm. Histologically, there was evidence of active epithelial proliferation with the formation of epithelial "pearls" and numerous mitoses. The stroma was scanty, the surrounding tissues were not invaded, and metastases were not found. After 16 months of observation the tumor appeared to be decreasing in size. A biopsy was taken and pieces transplanted to other fish; none of the transplants grew; no details were given.

THOMAS, 1930: In 2 soles *Solea solea* similar tumors were found behind and below the opercular opening on the pigmented side of each fish. Each of the tumors measured about 35 x 20 x 7 mm.; they were sessile and grossly lobulated; the overlying skin was devoid of scales. Massive pegs of epithelium with intact basement membrane extended deep into the subcutaneous tissue. Roentgenograms revealed that the adjacent bone was not involved. The author believed that these tu-

mors were due to chronic irritation of the postopercular skin by sand particles drawn into the mouth and expelled through the gill vent in the act of breathing.

THOMAS and OXNER, 1930: A tumor was located on the lower lip in each of 3 eels *Anguilla anguilla*. The lesions showed simple epithelial proliferation with a tendency to reduplication of the basal cell layer; the basement membrane was preserved.

THOMAS, 1932: A 32 cm. brown trout *Salmo trutta* bore an irregular tumor at the level of the posterior margin of the operculum. It measured 22 x 11 x 5 mm., was not sharply demarcated, and in both gross and microscopic structure was a typical papilloma.

COATES, COX and SMITH, 1938: A tumor appeared in the right mid-dorsal region of a six foot adult electric eel *Electrophorus electricus* caught in the Amazon river basin and maintained in good health in a fresh water tank at the N. Y. Aquarium for a period of more than four years. The growth began as a small elevation of the skin, pinkish-gray in color and a few millimeters in diameter. Its maximum size of 2 x 2.5 cm. was reached in about ten weeks, when it was excised. No recurrence took place in the remaining year and a half of life. The growth was a grape-like pedunculated mass composed of squamous epithelium commingled with very many mucous cells. The epithelium was supported by a central narrow fibrous core of connective tissue.

ADENOMA

Adenomas have been reported in 11 species of fish (Table 2). In 3 species the neoplasms involved structures which have no exact counterpart in man, namely, the parabranchial bodies. These are two small, often quite rudimentary organs, which lie in front of the true gills, and, like them, are hidden from view by the opercula or gill covers. They are composed of an extremely vascular tissue; the numerous capillaries are surrounded by large cells with clear or coarsely granular acidophilic cytoplasm. The function of the parabranchials is unknown; some investigators consider them to be endocrine organs.

Tumors of these structures have been observed in codfish *Gadus morhua* from St. Pierre and Miquelon, and in 2 kinds of coalfish from the Pacific. In the coalfish *Pollachius brandti* and *Theragra chalcogramma* in which the normal parabranchials vary in weight from 0.1 to 0.4 gm., tumors of 9 and of 16 gm., respectively, have been described (199). The tumor cells formed alveolar masses supported by a scanty but richly vascular stroma. The cells resembled those of the normal parabranchial bodies, having a clear cytoplasm when they were adjacent to the blood vessels, but with a granular cytoplasm when more centrally located. The nuclei were large and vesicular; mitoses were common. Necrosis of the densely packed cells in the central part of the alveolar mass led to the formation of pseudoacini. Usually the tumors were not well delimited and infiltrated the neighboring tissues. The investigators who studied these growths at first hand fail to agree as to their character. Takahashi (199) considered them to be adenocarcinomas; Peyron and Thomas (150) believed that they were benign or, at most, of low-grade malignancy.

Besides these tumors, adenomas have been reported in various organs of 8 other species of fishes. The tissue of origin was the liver in 3 species, and the kidney, intestine, and ovary in the others. Most of these tumors were solitary; some attained a very large size, the tumor reported by Plehn in the liver of a trout was twice the size of a man's fist.

Biesele (13) studied the chromosomes in an ovarian tumor of a goldfish. Unfortunately no histologic data are given and the author speaks of the growth as an "adenoma or adenocarcinoma." The average volume of the neoplastic chromosomes was twice that of chromosomes in the cells of a healing skin wound and in the nongerminal cells of a normal ovary. The normal nuclei never possessed more than 4 nucleoli, whereas 8 were found in the tumor cell nuclei. Biesele concluded that the chromosomes of the goldfish tumor must be regarded as diplochromosomes.

An adenoma of the catfish *Ameiurus nebulosus*, which has not previously been described, occurred in the right kidney of a female whose total length

TABLE 2: ADENOMA

Species	Site	Author
Sand shark *Prionace glaucus*	Liver	Schroeders, 1908
Trout *Salmo trutta*	Liver	Plehn, 1909
Ling *Molva molva*	Ovary	Johnstone, 1915
Codfish *Gadus morhua* (2)	Parabranchial body	Peyron & Thomas, 1929
Coal Fish *Pollachius brandti* (2)	Parabranchial body	Takahashi, 1929
Coal Fish *Theragra chalcogramma*	Parabranchial body	Takahashi, 1929
Purple Trout *Salmo mykiss*	Intestine	Thomas, 1931a
Rainbow Trout *Salmo gairdnerii*	Liver	Haddow & Blake, 1933
Platyfish *Platypoecilus maculatus* (several)	Kidney	Jahnel, 1939
Goldfish *Carassius auratus*	Ovary	Biesele, 1943
Catfish *Ameiurus nebulosus*	Kidney	Lucké & Schlumberger (not published)

was 35 cm. The growth measured 8 x 8 x 6 mm., was pale gray, and sharply delimited, though not encapsulated; its cut surfaces were moist but not bloody. It was composed of acini that were irregular in size and shape, and often greatly dilated. The lining epithelial cells varied from low cuboidal to tall columnar elements; their nuclei were large, vesicular, and had prominent nucleoli; mitotic figures were uncommon. Papillary projections extended into some of the cystic spaces; the scanty stroma was poorly vascularized. This tumor was found in a fish which also had massive epitheliomas of the lip and mouth.

Abstracts of other reported cases of adenoma

SCHROEDERS, 1908: Multiple adenomas were found in the liver of a shark *Prionace glaucus* caught in the Black Sea. The surface of the liver was covered with whitish, spherical nodules ranging in size from a pin-head to a walnut. Similar nodules were found scattered throughout the substance of the liver. On section the nodules were firm and pale yellow. Histologically, the neoplastic cells resembled normal liver cells except that they were smaller and contained less fat. The transition from normal cells to tumor cells was gradual.

JOHNSTONE, 1915: One ovary of a ling *Molva molva* was occupied by a mass consisting of cystic spaces with walls of varying thickness. On section, a clear, glairy fluid was found in the cysts. Some areas of fibrosis were also present in this lesion which may be classified as a papillary cystadenoma of the ovary.

PLEHN, 1924: The liver of a lake trout *Salmo trutta* was the site of a mass of cysts filled with clear fluid or gelatinous material. The largest of the cysts measured 15 cm. in diameter; the entire tumor was twice as large as a man's fist. Between the cysts were remnants of normal liver, strands of connective tissue, and isolated bile ducts. The growth was regarded by Plehn as a cystadenoma of the liver.

PEYRON and THOMAS, 1929: The authors observed tumors of the parabranchial bodies in two codfish *Gadus morhua*. In one, a large tumor, 4 x 3 x 1.5 cm., occurred on the right side, elevated the operculum, and protruded beyond its posterior margin; the gills were compressed. In the other case the tumors were bilateral and the size of a cherry. The histologic appearance has been discussed above.

TAKAHASHI, 1929: In two specimens of coalfish *Pollachius brandti* each parabranchial body was the seat of a tumor. In both, the growths on the right were the size of a small hen's egg, those on the left were twice as large. In another variety of coalfish *Theragra chalcogramma* a large tumor was present in the left parabranchial body. This tumor was soft in consistency, poorly circumscribed, and contained many cystic and necrotic areas. The surface was ulcerated. The histologic structure was as already described.

THOMAS, 1931 a: In a purple trout *Salmo mykiss*, 25 cm. in total length, the author found a broadly sessile, hemispheric tumor in the intestine midway between stomach and anus. It measured 20 x 18 x 8 mm., was gray-white and moderately firm; there was no ulceration of the surface. Histologically, the entire thickness of the intestinal wall was infiltrated by the cystic neoplasm. The lining epithelium of the cysts was stratified, the cells basophilic with occasional abnormal mitoses. Blood vessels were not numerous; collections of lymphocytes were abundant.

HADDOW and BLAKE, 1933: The authors described a hepatoma found near the anterior margin of the liver of a 4 to 5 year old rainbow trout *Salmo gairdnerii*. It was spherical, about 1.5 cm. in diameter, and paler and more firm than the surrounding hepatic tissue. No metastases were present. Microscopically the tumor was composed of actively proliferating polyhedral cells, only slightly different from the parent liver cells. The arrangement of the cells resembled that of normal liver, except for the presence in the tumor of an abundant fibrous stroma. Although the adenoma was not encapsulated, it had not infiltrated adjacent structures.

JAHNEL, 1939: This author notes the frequent occurrence of cystic adenomas of the kidney in the tropical aquarium fish *Platypoecilus maculatus*. No further details are given.

DENTAL TUMORS

Teeth are cutaneous appendages; in mammals they may be regarded as modified papillae of the oral mucosa. In elasmobranch fishes, such as sharks and rays, the small placoid scales, which give the rough granular feel to the skin of these animals, represent phylogenetically the most primitive teeth of vertebrates. Each scale has a pointed tooth-like shape; its base is embedded in the dermis, its apex protrudes above the surface. A central vascular connective tissue pulp is surrounded by a layer of odontoblasts which form the hard dentin. The free surface is coated by enamel, a product of modified epithelial cells.

In Table 3 is given a summary of dental tumors which have been observed in 4 species of fishes.

A neoplasm of "cutaneous teeth" was reported as a "dermal odontoma" by Ladreyt (108). The tumor,

TABLE 3: DENTAL TUMORS

Species	Site	Author
Trout—species not given (2)	Dental plates & hyoid bone	Plehn, 1912
Croaker *Micropogon opercularis*	Upper dental plate	Roffo, 1925
Haddock *Melanogrammus aeglifinus*	Maxilla	Thomas, 1926
Cat shark *Scyllium catulus*	Skin	Ladreyt, 1929

a cylindrical mass, measuring 3 x 1.2 cm., arose in the skin near the copulatory appendages of a cat shark *Scyllium catulus*. It was very firm, and had a small, very vascular cavity at its distal end. Histologically, a thick layer of cutaneous epithelium covered a more or less homogeneous mass, which the author believed represented enamel or dentin. The cavity noted grossly was occupied by greatly dilated blood spaces that appeared angiomatous.

Corresponding more closely to odontomas of man are the lesions described by Plehn (160) in the jaws of two fresh-water trout. In both cases the tumors consisted of large congeries of atypically formed teeth. Innumerable, more or less confluent, nodular tumors, had developed from all the tooth-bearing surfaces, *viz.*, both dental plates, the hyoid bones and, in one case, the vomer. As the author showed in a photograph, the tumors had attained such a large size that the fishes could not close their mouths, thus making feeding impossible.

Another example of an odontoma was reported by Roffo (172) in a marine fish, the croaker *Micropogon opercularis*. The tumor formed a firm, pale mass on the upper dental plate. Histologically it consisted of tooth germs irregularly scattered through a connective tissue stroma. The paper is accompanied by a number of excellent figures which show gross appearance, roentgenograms, and histologic structure of the neoplasm.

Only one case of adamantinoma has been recorded, by Thomas (202). The tumor formed a mass, 2.5 x 1 cm., that had infiltrated the maxilla and protruded into the oral cavity of a haddock *Melanogrammus aeglifinus*. Histologically it corresponded in all details to the cystic adamantinomas of man.

EPITHELIOMA (EPIDERMOID CARCINOMA)

Some of the neoplasms reported in the literature as epithelioma are only questionably malignant. In this review we have placed certain tumors which were reported as epitheliomas under the heading of papilloma because of their structure and absence of invasion. There remain a considerable number of tumors of pavement epithelium that are invasive or exhibit other evidence of malignancy, which properly may be regarded as epidermoid carcinomas. They have been observed in 12 different species of fishes (Table 4). The tissues of origin were the skin, the lips or oral mucosa, and, in a single case, the urinary bladder. In general, these tumors had the structure of mammalian epidermoid carcinomas; in some cases, as in that of Bashford, Murray and Cramer (6) the resemblance to squamous cell carcinomas in mammals was reported as striking. It must be remembered, however, that the epithelial covering of fishes does not keratinize, hence the typical cornified "pearls" of mammalian epitheliomas are not formed, although nonkeratinized collections of concentrically arranged cells are frequently seen.

Several of the tumors under discussion resembled papillomas, in their general appearance, but differed in that they sent long, usually ill-defined pegs of solidly packed cells into the corium or deeper tissues. Thus, in the case reported by Murray (141) the tumor invaded the underlying skeletal muscle. None had metastasized at the time of examination.

The lips and oral mucosa are the most common sites of epitheliomas in fishes; neoplasms have been observed in these locations in 7 species. The first record of an epithelioma in a fish reported by McFarland (136), deals with such a tumor. A lobulated, papillary growth, 4 cm. in diameter, involved the lower lip and adjacent mucosa of a catfish *Ictalurus catus*; multiple small nodules were present on the upper lip and the neighboring skin. Histologically the neoplasm consisted of a fungoid mass which was composed of epithelial cells supported by a delicate vascular stroma. The larger tumor was definitely invasive. The tumor reported by Clunet

TABLE 4: EPITHELIOMA (EPIDERMOID CARCINOMA)

Species	Site	Author
Catfish *Ictalurus catus*	Both lips	McFarland, 1901
Carp *Cyprinus carpio* (2)	Skin of head	Dauwe & Pennemann, 1904
Carp *Cyprinus carpio*	Not given	Bashford, Murray & Cramer, 1905
Stickleback *Spinachia spinachia*	Skin of trunk	Murray, 1908
Tench, *Tinca tinca* (2)	Lips	Fiebiger, 1909a
Carp *Cyprinus carpio*	Fins & operculum	Fiebiger, 1909a
Goldfish *Carassius auratus*	Bladder	Plehn, 1909
Barbel *Barbus vulgaris*	Lower lip	Clunet, 1910
"LeNez" *Chondrostoma soetta*	Oral mucosa	Mazzarelli, 1910
Croaker *Pogonias chromis* (2)	Lips	Beatti, 1916
Whiting *Merlangus merlangus*	Mandible	Johnstone, 1924
Codfish *Pollachius virens*	Lower lip	Williams, 1929
Biajaca *Cichlasoma tetracanthus*	Skin about orbit	Puente-Duany, 1930
Catfish *Ameiurus nebulosus* (166)	Lips, oral mucosa	Lucké & Schlumberger, 1941

(31) was located on the lower lip of a barbel *Barbus vulgaris*. It was the size of a hazel-nut and had infiltrated the floor of the mouth; structures suggestive of epithelial pearls were present and mitoses were numerous.

Fiebiger's report (47) of epitheliomas on the lips of two tench *Tinca tinca* is of interest in that the tumors occurred in fish from the same pond. The evidence of malignancy is not certain, for the author states that a fairly well preserved basement membrane surrounded the extensive epithelial pegs. In one of Beatti's (7) two cases of epithelioma in croakers, the basement membrane was intact in most regions, though in some areas epithelial pegs penetrated deeply. In the other this was more marked, and invasion of subcutaneous tissue had taken place.

We ourselves have been studying a transplantable epithelioma of the lip and mouth of catfish *Ameiurus nebulosus* taken from streams near Philadelphia (122). During a period of 2 years, 166 live tumor-bearing fish were obtained.* Since our previous publication approximately 100 additional cases have been studied. This neoplasm usually occurs as a solitary or multiple, large, red, fleshy mass upon the lips or dental plates (Figs. 8–11); less often it involves other parts of the mouth or the skin. In our series the lips and dental plates were affected in all but 6 cases. All of the neoplasms are grossly similar. They are broadly sessile, with a smooth or coarsely nodular surface; in consistency they are firm and resilient. Most of the tumors average from 1 to 2 cm. in diameter, but some are so massive as to prevent closure of the mouth (Fig. 10). In approximately one-half of the cases, both lips or dental plates are involved. In 53, or 60 per cent, of 89 fish the tumors were in direct apposition (Figs. 9 and 11). Histologically the tumors consist of closely packed masses of columnar or polyhedral cells, often growing as papillary pegs supported by a delicate richly vascular stroma (Fig. 12). The smaller tumors grow outward, with little sign of invasion. Larger growths commonly push broad solid pegs of epithelial cells deep into the subjacent tissues; in some, these pegs are bounded by a well preserved basement membrane, but in the more massive tumors they are definitely invasive, and flame-shaped processes of loosely arranged cells extend far into the subjacent tissues (Fig. 13). In the more invasive tumors emboli of neoplastic cells are often found, though no metastases have been observed. It seems evident that the small, outward growing tumors are early stages of a neoplastic process which later assumes a more malignant character.

It has been possible to study living tumors almost from the time of their inception. Tumor-bearing fish were maintained under laboratory conditions for periods up to 9 months; during this time we observed that growth of most of the neoplasms was relatively slow but progressive. The inception and development of appositional growth was studied by direct microscopic examination of the mucosal surfaces. The earliest evidence of neoplastic change was the establishment of a more or less circumscribed patch of hyperemia on the mucosa. At this time the mucosal surface was smooth and showed no signs of proliferation; after approximately two weeks the patch became slightly elevated; one or two months later the local thickening had progressed to the formation of a definite tumor.

Detailed microscopic study of the blood vessels *in vivo* brought out the fact that they undergo profound alterations during the development of the tumor. At the site of future neoplastic growth they gradually form irregular, wide meshed networks that contrast sharply with the small, uniform capillary loops of the adjacent normal mucosa. The caliber of the blood channels in the neoplastic zone varies greatly; some are dilated and bear saccular expansions; others are constricted (Figs. 14 and 15). Many of the proliferating vessels have thick walls, whereas others are thin and delicate. In brief, the neoplastic growth is preceded and accompanied by a striking vascular reaction; the number, arrangement, and structure of the vessels are conspicuously atypical. The relation of blood-vessels to tumor growth has previously been investigated mainly in fixed tissue; the catfish epithelioma provides material for such an investigation in the living animal.

Abstracts of other reported cases of epithelioma

DAUWE and PENNEMANN, 1904: Each of two very large, old aquarium carp *Cyprinus carpio* developed a tumor on the dorsum of the head, in front of the eyes. Microscopically the lesions were identical and consisted of squamous epithelial cells that formed long pegs containing central areas of necrosis. The authors regarded the tumors as squamous cell carcinomas.

BASHFORD, MURRAY and CRAMER, 1905: These authors mention an epithelioma in a carp *Cyprinus carpio* but do not give the location. They state that the tumor presented a striking resemblance to squamous

* Many of the tumor-bearing fish and several hundred normal fish which were used for experimental purposes have been furnished us through the kindness of Mr. C. R. Buller, Chief Fish Culturist of the State Fish Hatchery, at Bellefont, Pennsylvania, John J. Wopart, Jr., Superintendent of the State Fish Hatchery at Torresdale, Pennsylvania, and the late Dr. Robert O. Van Deusen, Director of the Philadelphia Aquarium. We wish to express our grateful appreciation to these gentlemen for the aid and advice given.

cell carcinoma of mammals. Multiple inoculations were made into 6 carp with negative results.

MURRAY, 1908: In a male stickleback *Spinachia spinachia* a flat cone-shaped tumor was located on one side of the tail. The tumor, histologically a squamous cell carcinoma, arose from the skin and invaded the myotomes of the affected side, penetrating to the vertebrae. The surface was ulcerated, the center necrotic.

FIEBIGER, 1909 a: An epithelioma was found on the lips of two tench *Tinca tinca,* both from the same pond. One bore a tumor at the angle of the mouth on the right that spread to both upper and lower lips. In the other fish the neoplasm involved the entire upper lip and extended backward on the left to the orbit. The surface of each tumor was nodular, containing collections of pigment cells and visible blood vessels. Histologically there was pronounced epithelial hyperplasia, but a fairly well preserved basement membrane surrounded the epithelial pegs. Mucous cells and clavate cells were present near the center of the cell masses.

FIEBIGER, 1909 a: In a carp *Cyprinus carpio* multiple wart-like masses the size of peas had almost destroyed the right pectoral fin. They were also present on the ventral and dorsal fins, and on the right operculum. Histologically the tumors were malignant papillary epitheliomas that invaded the corium and contained many multinucleate giant cells.

PLEHN, 1909: The abdomen of a goldfish was greatly distended by a dilated urinary bladder, the outlet of which was obstructed by a tumor. Histologically it was composed of neoplastic cells that tended to form alveoli and had penetrated deeply into the subjacent tissue.

MAZZARELLI, 1910: A 4.5 x 3.5 cm. tumor arose from the pavement epithelium of the mouth of a "Le Nez" *Chondrostoma soetta.* The flattened epithelial cells had large nuclei; some mucous cells were present. Local liquefaction necrosis and cyst formation was observed in the epithelial pegs.

BEATTI, 1916: Tumors were found on the lips of 2 croakers *Pogonias chromis.* On the upper lip of one there was a small cauliflower-like growth; another papillomatous tumor was located on the forehead. The stratified epithelium as well as the connective tissue of the cutis were hyperplastic; the basement membrane in most regions was intact, though at one point epithelial pegs penetrated deeply. In the second fish the lower lip was the site of a firm, diffuse swelling. The histologic appearance of malignancy was more marked in this case than in the former; the epithelial pegs invaded the subcutaneous tissue more deeply. The tumors may be classified as papillary epitheliomas with early but definite signs of invasion.

JOHNSTONE, 1924: In a whiting *Merlangus merlangus,* on the right side of the mandible and extending a short distance into the mouth, was a 1 x 1 cm. tumor. Histologically it was a typical epithelioma with extensive proliferation of epithelial pegs but an intact basement membrane.

WILLIAMS, 1929: The author reported a bilobed growth which had spread over the anterior portion of the lower jaw of a codfish *Pollachius virens* caught near the coast of Iceland. It may have arisen in the mucous cells which are abundant in the integument of fish. Evans, of the Pathology Department in Liverpool, thought that it corresponded to human epithelioma adenoides cysticum.

PUENTE-DUANY, 1930: A periocular tumor was found in the orbit of a small Cuban fresh water fish, the biajaca *Cichlasoma tetracanthus;* though the neoplasm had produced exophthalmos, the overlying skin was intact. Histologically, the epiderm was thickened, epithelial pegs penetrated into the dermis. In some areas these pegs fused with masses of clear, polyhedral cells having small round nuclei. These cells were the characteristic feature of the tumor; their origin was uncertain. The growth was recorded as an epithelioma, possibly arising on an inflammatory basis.

ADENOCARCINOMA

Malignant tumors of gland-cell origin are the predominant cancers in man. By contrast, but few examples, 7 in all, have hitherto been reported in fishes. This fact does not permit us to conclude that this kind of cancer is uncommon in fishes; it may mean that an adequate search has not yet been made. This supposition is the more plausible because most adenocarcinomas originate in the viscera, and not on the body surface as do the epitheliomas.

The tumors occurred in 6 different species (Table 5). All but one of the tumors were located within the body cavity. As shown in the table, in 2 cases the tumors had their origin in the kidney of eels *Anguilla anguilla*; both neoplasms were relatively large 7 x 3.5 cm., and both were invasive and destructive. A tumor in a red tai *Pagrosomus major,* arose behind the right kidney, but had no direct connection with it. It was large, 9 x 3.5 cm., uniformly

TABLE 5: ADENOCARCINOMA

Species	Site	Author
Eel *Anguilla anguilla*	Kidney	Schmey, 1911
Eel *Anguilla anguilla*	Kidney	Plehn, 1924
Coalfish *Pollachius brandti*	Glandula digitiformis	Takahashi, 1929
Coalfish *Theragra chalcogramma*	Mouth & operculum	Takahashi, 1929
Red Tai *Pagrosomus major*	Body wall	Takahashi, 1929
Pike *Esox lucius*	Ovary	Haddow & Blake, 1933
Moray *Muraena helena*	Palate	Ladreyt, 1935

soft, pale gray, and invaded the body wall. The component cells were cuboidal and had large spherical nuclei; the stroma was abundant. Takahashi (199) regarded it as an adenocarcinoma of unknown origin.

The ovarian tumor in a pike *Esox lucius* reported by Haddow and Blake (68) was a soft mass, 7 x 3 cm., near the anterior pole of the left ovary; several smaller nodules accompanied the main tumor. Microscopically, the structure was that of an adenocarcinoma, apparently derived from germinal epithelium.

The cancer of the digitiform or rectal gland in the coalfish *Theragra chalcogramma* reported by Takahashi (199) is of interest because of its extensive metastases and its origin in a structure having no homologue in man or the higher vertebrates. This gland is a short tubular body, the duct of which opens into the lower end of the intestine; its function is unknown. The mass was firm, coarsely nodular, grayish white, and as large as the head of a child. Histologically it was an adenocarcinoma that contained cystic spaces partly filled by papillary projections. Metastases and secondary tumors due to direct extension were present in the liver, stomach, intestine, spleen, urinary and swim bladders.

Abstracts of other reported cases of Adenocarcinoma

SCHMEY, 1911: A retroperitoneal mass 7 x 3.5 cm. was found in an eel *Anguilla anguilla* approximately 3 years old. The tumor could be readily freed from the abdominal musculature, but could not be separated from the left kidney. Microscopically it consisted of irregularly arranged tubules and cysts which passed over into normal renal substance without sharp demarcation.

PLEHN, 1924: The abdomen of an eel *Anguilla anguilla,* 53 cm. in length, was greatly distended by a tumor 7 x 3.5 cm. The lesion was apparently malignant, having destroyed the posterior half of the kidney and invaded the anterior portion, but no metastases were found. No histologic description accompanied the report.

TAKAHASHI, 1929: At the left angle of the mouth of a coalfish *Theragra chalcogramma* was a tumor that had extended into the skin of the operculum on the same side. The neoplastic epithelial cells were grouped to form alveoli and invaded the adjacent structures. The tissue of origin was not identified.

LADREYT, 1935: The palate of a moray *Muraena helena* was the site of a large tumor which had its origin in the glands scattered throughout the mucosa. The malignancy of the tumor was manifested by partial absence of a basement membrane, atypical structure of the cells, and extensive local invasion. Metastases were not found. It is well known that the bite of Morays is poisonous, due to the secretion of venom by the mucous glands in the palate. Tests showed that the venom of the cancerous glands was less active than that of the normal ones, but the effects it produced—hyperemia and cellular degeneration—were qualitatively the same.

THYROID "TUMORS"

No tumor or tumor-like lesion of fishes has received more attention than that of the thyroid gland. Such "tumors" are most frequently observed in trout reared in hatcheries and less often in other species living in ponds or aquaria (98, 131, 178, 190, 194) (Table 6). Attention was first called to this condition by Bonnet (14) who described an epidemic that destroyed over 3,000 lake trout *Salmo lacustris* in a fish hatchery between February and June of 1883. All had tumors at the ventral junction of the gills.

Scott (182), who observed enlargement of the thyroid of brook trout *Salvelinus fontinalis* reared in a single pond, was the first to identify it as "carcinoma" (1891). The lesion began as a local hyperemia of the oral mucosa followed by nodular roughening in the midline of the ventral wall of the pharynx just behind the tongue. The nodules increased in size, and ultimately filled the oral cavity, protruding beyond the opercula. On section the masses were found to be composed of distended irregular acini lined by cuboidal or columnar cells. Some of the alveoli were filled with colloid; the lumen of others was small and the cells arranged in papillary folds. Islands and acini of thyroid cells were found scattered throughout the adjacent tissues.

The view that these tumors are true cancers has been maintained by numerous investigators (25, 58, 78, 153, 156, 195). In 1914 Gaylord and Marsh published their monograph entitled "Carcinoma of the Thyroid in the Salmonoid Fishes" (53), based on tumors observed in several thousand trout at U.S. government fish hatcheries. A study of the histologic descriptions and many excellent photomicrographs reveals, however, that the large majority of the tumors are examples of hyperplasia and colloid storage, without evidence of malignant change. Nevertheless, it cannot be denied that among the thousands of goiters there probably were instances of adenocarcinoma. Gaylord and Marsh themselves reported two cases in which metastases were found. In one, the metastatic nodule was present in the wall of the rectum, where it was covered by intact rectal mucosa. In the other the metastasis was on the tip of the lower jaw and may merely represent aberrant thyroid tissue. This is made more likely in view of the studies of Gudernatsch (66) who pointed out

that the teleost thyroid is not encapsulated and that its follicles do not form a distinct organ but are scattered over a wide area. Although the main bulk of the thyroid develops about the stem of the ventral aorta between the first and second gill arches, groups of follicles are not uncommonly found among the adjacent muscle bundles and bone lamellae. This natural arrangement may be erroneously interpreted by the unwary as evidence of invasiveness.

In a series of papers published in 1910–1914 Marine and Lenhart (127–130) reported the results of their observations of this disease among brook trout in a large fish hatchery in Pennsylvania. They denied that the lesion is a carcinoma (128): "In view of the facts that young fish are more affected than old fish, that iodine stops the hyperplasia and causes it to return to the colloid state, that removal of the fish from the ponds to the open brook also effects a cure, that the clinical incidence of tumors is directly related to the water supply, and that the severity of the disease as determined by histological examination is likewise directly related to the water supply, we cannot accept the prevailing opinion that the disease is true cancer. On the other hand, we believe that the disease as studied by us is an extreme illustration of endemic goiter, the end stage of which is cretinism." The age incidence and development of the lesion was summarized by Marine (130): "It was found that the thyroid hyperplasia began in the fry as soon as feeding was instituted, and advanced overgrowth was present at the 4th month of extra oval life; that the overgrowth progressively increased to the stage of clinical detectability, as ascertained by the reddening of the pharyngeal floor over the thyroid area, about the tenth month in this hatchery; that visible goiters usually manifested themselves about the beginning of the second year, though they may be present as early as the sixth month, depending on the favorableness of conditions for overgrowth, and progressively increased during the second and third years; that older fish were more resistant and tended toward spontaneous recovery." Marine does not deny, however, that the hyperplasia of the thyroid may in some instances progress to actual carcinoma.

In a recent monograph on the cause of thyroid hyperplasia in animals and man, Duerst (40) expressed the belief that the functional demand made upon the thyroid is in inverse proportion to the oxygen tension of the air or, in the case of fishes, of the surrounding water. He points out that carp and tench require only 3 to 4 cc. of oxygen per liter of water, while trout require 7 to 8 cc. When the oxygen content of the water falls to 3 to 4 cc. per liter the young trout develop thyroid hyperplasia, at 1.5 cc. per liter they die of asphyxia; whereas carp and tench survive at a 0.5 cc. per liter level. The predominance of thyroid tumors in the *Salmonidae* may be related to the high oxygen demands of these fishes and the low oxygen tension present in the tanks of the fish hatcheries. The fact, noted by Marine, that the goiters occur particularly in young

TABLE 6: THYROID TUMORS

Species	Author
Lake trout *Salmo lacustris* (3,000)	Bonnet, 1883
Brook trout *Salvelinus fontinalis* (many)	Scott, 1891
Rainbow trout *Salmo gairdnerii* (3)	Gilruth, 1901
Amer. Salmon *Salmo salar*	Gilruth, 1902
Trout several species (several)	Plehn, 1902
Trout several species (many)	Pick, 1905
Trout several species (6)	Jaboulay, 1908
Lake trout *Salmo lacustris*	H. M. Smith, 1909
Trout several species (thousands)	Marine & Lenhart, 1910, 1911, Marine, 1914
Trout several species (thousands)	Gaylord & Marsh, 1914
Dogfish *Squalus sucklii*	Cameron & Vincent, 1915
Sea Bass *Serranus scriba* & *S. cabrilla* (5)	Marsh & Vonwiller, 1916
Barbel *Barbus fluviatialis* (several)	Schreitmüller, 1924
Trop. Aquar. Fish *Jordanella floridae* (several)	Schreitmüller, 1924
Catfish *Clarias dumerlii*	Schreitmüller, 1924
Porgy *Box vulgaris*	Johnstone, 1924
Rainbow trout *Salmo gairdnerii* (many)	Leger, 1925
Aquar. Fish *Danio albolineatus*	Klemm, 1927
Aquar. Fish *Haplochilus chaperi* (several)	Klemm, 1927
Trout species not given	Peyron & Thomas, 1930
Salmon species not given	Peyron & Thomas, 1930
Aquar. Fish *Rasbora lateristriata* (several)	Smith, Coates, & Strong, 1936
Aquar. Fish *Heterandria formosa* (several)	Smith, Coates, & Strong, 1936
Aquar. Fish *Rasbora lateristriata* (several)	Smith & Coates, 1937
Suckfish *Echeneis naucrates* (16)	Lucké & Schlumberger (not published)

fish, and tend toward spontaneous regression in older animals, may be related to the extensive involution which the thyroid normally undergoes when the fish reaches sexual maturity—at the age of 3 or 4 years in trout. The involution of the thyroid is compensated for, at least as concerns its oxygen regulating function, by an increase in the number of erythrocytes in the circulating blood (40).

The possible relation of low oxygen tension in water to thyroid hyperplasia in fish receives support from the work of Marsh and Vonwiller (131). These investigators found enlarged thyroids in five sea bass *Serranus scriba* and *S. cabrilla* that died in the aquarium of Naples. Since these fishes were kept in fresh sea water, in which the content of iodine is high, depletion of the latter cannot account for the occurrence of the goiters, which histologically resembled those of the *Salmonidae*.

Thyroid enlargements have been seldom observed in marine fishes hence their appearance in a group of shark suckers *Echeneis naucrates* is of interest. These fish attach themselves to the belly of a shark by means of a modified dorsal fin that has been transformed into a suction plate. They are not strictly parasitic, since they derive no nourishment from the body of their host; the latter merely serves as a vehicle to carry them to their food supply. Three of these fish measuring nearly 3 feet in length were caught off the coast of New Jersey and exhibited for several weeks at a seaside resort. They were then transferred to the Fairmount Park Aquarium in the City of Philadelphia, where we had an opportunity to study them. The fish were kept in a large tank of aerated sea water and fed a diet of fresh shrimp, oysters, clams, and chopped fish. Approximately 6 weeks after arrival, a pink translucent, coarsely lobulated tumor appeared on the floor of the mouth in one of the fish. The animal was subsequently sacrificed, at which time the tumor measured 4 x 3 x 2.5 cm. almost filled the oral cavity, and obstructed the free flow of water over the gills (Fig. 16). Microscopically the tissue proved to be thyroid, consisting in part of greatly dilated acini filled with marginally vacuolated colloid and lined by cuboidal or columnar epithelium, which formed occasional low papillary projections (Fig. 18). Alternating with the large colloid spaces were areas in which the acini were very small. Thyroid tissue was found between lamellae of bone (Fig. 17), but there was nothing to suggest that this represented an invasion of the bone. The histologic picture closely resembled the hyperplasia with extensive colloid storage frequently observed in non-toxic colloid goiters of man. Bits of tissue were inoculated into the anterior chamber of the eyes of three goldfish *Carassius auratus* and six large frogs *Rana pipiens*, but they grew in none and were rapidly resorbed.

A year later the second of these 3 fish had developed a goiter which in size and structure closely resembled the first. Three weeks thereafter 13 shark suckers, apparently free of goiters, were received from the New York City Aquarium and placed in the same tank with the one remaining shark sucker. Within 5 months, six of these fish showed marked enlargement of the thyroid (Fig. 19); after another 5 months all the fish were similarly affected. In all but one fish the goiters were confined to the oral cavity, measured between 1 and 3 cm. in diameter, and varied in color from pale pink to bright red. In the single remaining fish the thyroid had extended backward beneath each operculum, lifting that structure away from the side of the body. The animal rested upside down on the bottom of the tank, apparently to avoid further pressure on its gills and to facilitate breathing.

The etiology of the thyroid enlargement in these fishes is obscure. The oxygenation of the water appeared adequate. The iodine rich diet of shrimp, clams, and fish, which acted as a cure in the cases of goiter in trout reported by Marine, was apparently without effect. In none of the other marine fishes kept under identical conditions in the aquarium did goiters appear, nor were they known to occur in the fresh water fishes exhibited in the same building. The goiters of the shark suckers may be a response to some metabolic idiosyncrasy of these fish. This view is strengthened by the report of an official who stated that during his service of over twenty years he had frequently observed this lesion in the shark sucker, but never in any other fishes.*

Abstracts of other reported cases of thyroid tumors

GILRUTH, 1902: A five year old salmon *Salmo salar* and 3 rainbow trout *Salmo iridens* each bore a tumor the size of a walnut, that projected beyond both gill clefts and appeared to have started at the apex of the second branchial arch. On section the tumors were homogeneous, pale pink in color and soft in consistency. A fibrous capsule surrounded them, and connective tissue trabeculae extended from the capsule into the tumor parenchyma. The alveoli were irregular and lined by columnar or cuboidal epithelial cells. The tumors were well vascularized and frequent hemorrhages were present. Although the author identifies the mass as an endothelioma, it probably represents a simple hyperplasia

* We wish to express our grateful appreciation of the late Dr. Robert O. VanDeusen, superintendent of the Fairmount Park Aquarium, who placed these and many other interesting specimens at our disposal.

of the thyroid. The salmon was collected and described for Gilruth by H. C. Wilkie, the 3 trout by L. F. Cryson.

PLEHN, 1902: A preliminary report on "adenocarcinoma" of the thyroid in trout. The author discusses the normal anatomy of the gland in fishes and likens the pathologic changes in her specimens to adenocarcinomas of the thyroid in mammals.

PICK, 1905: The fishes studied by Pick had been bred in American fish hatcheries, and sent to him in Germany. Despite the variability of the histologic findings, Pick thought that the growths were unmistakable epitheliomas. He believed that primary simple endemic goiter undergoes a malignant transformation in consequence of some external deleterious influences.

JABOULAY, 1908: Six trout died following development of a malignant tumor of the thyroid. The author identified the tumors as adenocarcinomas that had invaded the surrounding tissues. He implicated hereditary factors as well as a contagious etiologic agent. The former opinion he supported by the fact that fish raised from eggs sent out of Germany suffered from the disease, whereas native trout under similar conditions remained well. Jaboulay believed that the infectious agent was a myxosporidium present in the water and taken into the digestive tract.

SMITH, 1909: A large tumor of the thyroid gland in a lake trout *Salmo lacustris,* was histologically a carcinoma of the alveolar type, not distinguishable from similar tissue of human origin.

CAMERON and VINCENT, 1915: The authors found a thyroid tumor in a dog fish *Squalus sucklii* which they considered histologically similar to those reported by Marine and Gaylord in trout. They pointed out that whereas in teleosts the thyroid is scattered and not encapsulated, in elasmobranch fishes it is definitely encapsulated and forms a compact organ. This point gives significance to their finding of invasion into the surrounding tissues. A quoted report by Adami suggests that histologically their specimen is a carcinoma.

SCHREITMÜLLER, 1924: The author described what he considered were examples of malignant neoplasia of the thyroid in *Jordanella floridae* and other oviparous and viviparous toothed carp, in barbels, and in a catfish (cited by Klemm).

JOHNSTONE, 1924: On the floor of the mouth of a porgy *Box vulgaris* on each side of the midline and in the region of the thyroid was a 2.5 x 1.3 cm. tumor which had lifted the operculum on that side. Histologically there were numerous large acini filled with colloid and lined by tall columnar cells.

LEGER, 1925: Thyroid tumors were found in rainbow trout *Salmo gairdnerii* at a hatchery in Grenoble. Leger states that most of them are adenomas, a few are adenocarcinomas, and he presents an example of the latter which had metastasized to the heart.

KLEMM, 1927: Tropical aquarium fish of the species *Haplochilus chaperi* (an oviparous toothed carp) appeared unwell when they had attained a length of 1.5 to 2 cm. Examination revealed that the opercula were lifted and breathing was rapid. On the pharynx a reddened, hemispherical mass could be seen, 2.5 to 3 mm. in diameter, having the histologic characteristics of a colloid goiter. A similar lesion was found in a specimen of *Danio albolineatus.*

PEYRON and THOMAS, 1930: The authors review the problem of thyroid tumors in fishes, especially the Salmonidae. Though inconclusive, they seem to favor the view that most are malignant neoplasms and include two cases of their own: one in a salmon, the other in a trout, and both typical of the many other goiters described in the literature.

SMITH, COATES and STRONG, 1936: These authors observed thyroid hyperplasia in 2 species of tropical aquarium fishes *Rasbora lateristriata* and *Heterandria formosa.* They believed that the tumors are benign adenomas; the discussion is brief.

SMITH and COATES, 1937: The authors present a detailed morphologic study of the normal and hyperplastic thyroid in a tropical aquarium fish *Rasbora lateristriata* that reaches a length of 3 cm. In the hyperplastic gland there is a great irregularity in the size and an increase in the number of follicles. Cross sections of the normal gland reveal not more than 10 follicles, whereas in the thyroid tumor these may number 3,000. Scattered collections of lymphoid cells were not uncommon in the tumor which also showed increased vascularity. It infiltrated the adjacent muscle, cartilage, and bone. The disease was usually fatal in two months, as the tumor destroyed and replaced the gill structures.

TUMORS OF MESENCHYMAL TISSUES

In this group are included: fibroma, myxoma, chondroma, osteoma, lipoma, leiomyoma, rhabdomyoma, hemangioma, osteo-sarcoma, lymphosarcoma, and sarcomas not further classified.

FIBROMA

The most common neoplasms of the supporting tissues of fishes are the benign and malignant tumors of the connective tissue proper. Fibroma has been reported in 18 species (Table 7). The subcutaneous tissue and trunk musculature are most frequently involved; however, fibromas have been found in the oral cavity, peritoneum, stomach and esophagus. Guglianetti (67) reported a slow growing retro-orbital tumor in a goldfish *Carassius auratus* that was under observation for 3 years. The right eyeball protruded, the pupil was greatly dilated, and the lens opaque. The tumor measured 3.5 cm. in diameter, was elastic and rather soft in consistency. Histologically the growth consisted of connective tissue that was rich in nuclei and contained several areas of necrosis.

Subcutaneous fibromas in goldfish have been described by several authors (45, 175, 226). They were often multiple and in one instance (226) involved several fishes in the same pool. An infectious

TABLE 7: FIBROMA

Species	Site	Author
Carp *Cyprinus carpio*	Peritoneum	Crisp, 1853
Carp *Cyprinus carpio*	Body cavity	Gervais, 1876
Lake trout *Salmo lacustris*	Subcutaneous	Eberth, 1878
Codfish *Gadus morhua*	Subcutaneous	Bland-Sutton, 1885
Codfish *Gadus morhua*	Stomach wall	Bland-Sutton, 1885
Thwaite shad *Alosa finta*	Wall of stomach	Ryder, 1887
Crucian carp *Carassius carassius*	Abdominal wall & mesentery	Plehn, 1906
Bream *Abramis brama*	Coelom	Plehn, 1906
Pike *Esox lucius*	Musculature	Plehn, 1906
Codfish *Pollachius virens*	Oral cavity	Fiebiger, 1909a
Codfish *Gadus morhua*	Esophagus	Williamson, 1909
Goldfish *Carassius auratus*	Orbit	Guglianetti, 1910
Sardine *Arengus pilchardus*	Peritoneum	Johnstone, 1911
Haddock *Melanogrammus aeglifinus*	Subcutaneous	Johnstone, 1911
Halibut *Hippoglossus hippoglossus*	Retroperitoneal	Johnstone, 1913
Halibut *Hippoglossus hippoglossus*	Trunk muscle (multiple)	Johnstone, 1914
Carp *Cyprinus carpio*	Mesentery	Ronca, 1914
Codfish *Gadus morhua*	Orbit	Johnstone, 1914
Goldfish *Carassius auratus* (several)	Subcutaneous	Wago, 1922
Plaice *Pleuronectes platessa*	Dorsal fin	Johnstone, 1922
Haddock *Melanogrammus aeglifinus*	Subcutaneous (multiple)	Johnstone, 1924
Turbot *Rhombus maximus*	Dorsal fin	Johnstone, 1924
Codfish species not given	Stomach wall	Johnstone, 1925
Sardine *Arengus pilchardus*	Peritoneum	Johnstone, 1925
Goldfish *Carassius auratus*	Skin (multiple)	Sagawa, 1925
Goldfish *Carassius auratus*	Back muscles	Eguchi & Oota, 1926
Halibut *Hippoglossus hippoglossus*	Subcutaneous, on head	Johnstone, 1926
Plaice *Pleuronectes platessa*	Subcutaneous, on head	Johnstone, 1926
Crucian carp *Carassius carassius*	Ovary	Freudenthal, 1928
Coalfish *Theragra chalcogramma* (2)	Belly wall	Takahashi, 1929
Rockfish *Sebastodes inermis*	Body wall	Takahashi, 1929
Codfish species not given	Wall of duodenum	Thomas, 1933a
Trop. Aquar. Fish *Rasbora daniconius*	Region of dorsal fin	Smith, Coates, & Strong, 1936
Brown trout *Salmo trutta*	Intra-abdominal	Kreyberg, 1937

agent could not be demonstrated, nor could the tumors be transplanted to other goldfish (45). The relationship of these growths to the subcutaneous fibrosarcomas of goldfish cannot be determined from the reports in the literature. A fibroma of the ovary in a related species, the carp *Carassius carassius* was reported by Freudenthal (52). The growth was nodular, white, and did not adhere to surrounding structures. Histologically the richly cellular parenchyma consisted of connective tissue, was not infiltrative, and contained no demonstrable parasites. Bits of the tumor were inoculated intraperitoneally into 2 other carp. One of these fish died on the following day; in the other no tumor tissue could be found when the animal was sacrificed 10 weeks later.

The first report of a soft tissue tumor in a fish was made by Crisp (33) at a meeting of the London Pathological Society in December, 1853. The neoplasm was a fibroma weighing 4 pounds and loosely attached to the peritoneum of a 10 pound carp. On section it was found to consist of dense, white bundles of fibrous tissue. The author called attention to the resemblance in origin and growth between this tumor and some of the tree fungi, pointing out that these excrescences, both in man and the lower animals, possessed a power of growth to some extent independent of the body to which they are attached.

Abstracts of other reported cases of fibroma

GERVAIS, 1876: A very large tumor was found near the ovaries in the abdominal cavity of a carp *Cyprinus carpio*. Histologically it was composed of connective tissue.

EBERTH, 1878: During a robbery at a fish hatchery in Switzerland, a large lake trout *Salmo lacustris* was injured by a blow on the head, but "the burglar failed to capture the tasty morsel." Though it bled from the wound, the fish was active and in a short time the wound had fully healed. At the site of the scar a nodular thickening developed which grew rapidly but remained covered by a smooth, intact layer of epithelium. Histologically the tumor was vascular, the tissue rather loose and somewhat gelatinous. In some areas the neoplasm resembled granulation tissue. The author identified it as a fibrosarcoma.

BLAND-SUTTON, 1885: In a codfish *Gadus morhua* a fibrous tumor lay in the loose tissue beneath the skin of the abdomen. The growth had undergone calcification.

BLAND-SUTTON, 1885: In the stomach of each of two codfish *Gadus morhua* was a pale gray tumor which on section was found to be composed of interlacing bundles of glistening white connective tissue.

RYDER, 1887: Described a fibroma near the pylorus of a thwaite shad (cited by Thomas, 1931 b).

PLEHN, 1906: A tumor the size of a hen's egg was attached to the peritoneum of a crucian carp *Carassius carassius* by a short pedicle. The mass was firm and had a smooth surface; the cut surface was pale pink. Histologically the tumor was a typical fibroma and was made up of interlacing bands of connective tissue. A few small areas of necrosis were present, no mitoses were found. Several smaller fibromas were scattered over the surface of the mesentery.

PLEHN, 1906: A firm tumor the size and shape of a hen's egg was found in the coelom of a bream *Abramis brama.* The neoplasm was composed of short spindle cells and fibers; no mitoses were found, no inflammatory cell infiltrates were present.

PLEHN, 1906: A pink tumor 3 x 2.5 cm., was present in the trunk muscles of a pike *Esox lucius.* The parenchyma was composed of connective tissue cells arranged in bundles, with many fat cells scattered among them. Mitoses were uncommon; occasional giant cells with peripheral nuclei were present. The author called the tumor a lipofibroma.

FIEBIGER, 1909 a: Arising from the oral mucous membrane of a codfish *Pollachius virens* was a tumor the size of a pigeon egg that consisted of bundles of connective tissue covered by a thick layer of epithelium.

WILLIAMSON, 1909: The author reported a fibroma of the esophagus in a codfish *Gadus morhua.*

JOHNSTONE, 1911: A fibroma 5.8 x 3.3 x 2.3 cm. was attached to the peritoneum in the region of the caecum of a sardine *Arengus pilchardus.* The surface was nodular and yellow-white in color. Histologically the tumor was composed of interlacing bundles of connective tissue.

JOHNSTONE, 1911: Behind the operculum of a haddock *Melanogrammus aeglifinus* was a well encapsulated tumor 7 x 6 x 6 cm. made up of bundles of mature connective tissue.

JOHNSTONE, 1913: A retroperitoneal fibroma weighing 2.5 kg. was found in a halibut *Hippoglossus hippoglossus.* The tumor, which was soft and friable and had a smooth, lobulated surface, was made up of bundles of connective tissue.

JOHNSTONE, 1914: Multiple fibromas were found in the trunk muscles of a halibut *Hippoglossus hippoglossus.* They were well encapsulated and could readily be shelled out.

JOHNSTONE, 1914: In the left orbit of a codfish *Gadus morhua* was a firm gray-white tumor 7 x 6 cm. The overlying skin was intact; the eye was displaced posteriorly and downward. The neoplasm was well encapsulated and consisted of interlacing bundles of connective tissue.

RONCA, 1914: A tumor the size of a hen's egg arose in the mesentery of a carp *Cyprinus carpio.* White in color, it was soft in consistency and was made up of interlacing bundles of connective tissue.

WAGO, 1922: Multiple tumors, 0.5 cm. in diameter, were scattered over the body surface, fins, and eyes of a goldfish *Carassius auratus.* Spheroidal or polypoid in shape, they were smooth, not covered by scales, and firm in consistency. The cut surface was red and translucent. Histologically there was an abundance of delicate connective tissue and fusiform fibroblasts mingled with irregularly shaped cells widely separated from one another. No inflammatory cell infiltrates were seen. Though several fish with similar lesions were found in the same garden pool, an infectious agent could not be demonstrated. The author identified the tumor as myxofibroma.

JOHNSTONE, 1922: On the pigmented side of the dorsal fin of a plaice *Pleuronectes platessa* was a firm spherical tumor 3.5 x 3 cm.; on microscopic examination it proved to be a typical fibroma.

JOHNSTONE, 1924: Scattered over the trunk of a haddock *Melanogrammus aeglifinus* were local thickenings in the corium. Each of these measured about 5 mm. in diameter, and was covered by normal epidermis. The author identified the lesions as fibromas.

JOHNSTONE, 1924: On the dorsal fin of a turbot *Rhombus maximus* was a tumor that measured 1 x 1½ inches. Histologically it was a typical fibroma.

JOHNSTONE, 1925: An immature female sardine *Arengus pilchardus* bore a tumor 9 x 4 cm., which apparently arose in the peritoneum. The viscera were intact. Microscopically the tumor consisted of irregularly arranged bundles of connective tissue abundantly supplied with blood vessels.

SAGAWA, 1925: The author found multiple fibromas on the skin of a goldfish *Carassius auratus.* The tumors were firm and elastic, yellow-gray, sharply circumscribed, and varied from the size of a millet seed to that of a pigeon's egg. Histologically they were composed of young connective tissue which, however, did not take van Gieson's stain. Myelinated nerve fibers were also present. (These lesions may have been neurofibromas.)

JOHNSTONE, 1925: The cardiac end of the stomach of a 16 pound codfish bore a tumor 15 x 10 cm. On section it was white, glistening, and firm in consistency. Histologically the neoplasm was a fibroma.

JOHNSTONE, 1926: On the nonpigmented side of the head of a halibut *Hippoglossus hippoglossus* was a mass 8 inches in diameter; the overlying skin was intact. The tumor weighed 3 pounds, shelled out readily, was well encapsulated, and histologically was a typical fibroma.

JOHNSTONE, 1926: Two tumors were present on the head of a plaice *Pleuronectes platessa.* One on the lower border of the operculum measured 4½ x 3½ inches, the other was smaller, grew from the anterior border of the right orbit, and partly covered the eye. The overlying skin was intact. Both neoplasms consisted of bundles of connective tissues; few vessels were present.

EGUCHI and OOTA, 1926: A firm elastic tumor

was found on the back of a goldfish *Carassius auratus* near its head. Though no scales were present in the overlying skin, ulceration was absent. Histologically the lesion was composed primarily of spindle-shaped cells. Attempts to transplant the tumor to other goldfish were unsuccessful.

TAKAHASHI, 1929: Embedded in the trunk muscles of a coalfish *Theragra chalcogramma* was a gray-white tumor, 4 x 3.5 x 3.2 cm. The overlying skin was intact. The tumor parenchyma consisted of spindle and round cells; there was no evidence of infiltration. Bits of the neoplasm were inoculated into 18 crucian carp; in none was there any growth.

TAKAHASHI, 1929: A cherry-sized, firm uniformly white tumor was found in the body wall near the caudal fin of a rockfish *Sebastodes inermis*. Histologically it consisted of bands and whorls of connective tissue.

THOMAS, 1933 a: A pedunculated fibroma 9 x 7.5 cm. was found attached to the mucosa of the intestine, near the pylorus. The host was a 9 year old codfish.

SMITH, COATES and STRONG, 1936: A small tropical aquarium fish *Rasbora daniconius* bore a sharply circumscribed, encapsulated, black tumor in the region of the dorsal fin. It consisted of loosely arranged interlacing bundles of elongated connective tissue cells.

KREYBERG, 1937: In a brown trout *Salmo trutta* a slightly lobulated, firm, pink tumor was discovered in the abdominal cavity. The mass measured 18 cm. in diameter, and was attached to the serosa of the intestine by a narrow pedicle. The parenchyma was composed of fibrous tissue, some of which was necrotic. No mitoses were present.

SARCOMA (UNCLASSIFIED)

The intermutability of mesenchymal tissues, and the complexity of tumors arising from these tissues, render their classification difficult (195a, 231a). Moreover, the boundary between non-malignant and malignant mesenchymal tumors is frequently vaguer than in epithelial tumors. Hence it is quite possible that some tumors here listed as sarcomas actually belong among the fibromas. It seems best therefore to list these two groups in proximity. No attempt has here been made to subdivide the sarcomatous tumors, other than separating the osteosarcomas and lymphosarcomas.

The sarcomas form the largest group of piscine neoplasms, having been observed in 31 species (Table 8). The predilection of the connective tissues to undergo malignancy is not unique to fishes; it also occurs in certain mammals such as the rat (24). The majority of the tumors listed in Table 8 had their origin in the dermis or subcutaneous tissues. However, this may not represent the true relative organ incidence since such growths would be more likely to attract attention than neoplasms of the viscera.

Of particular interest are a number of sarcomas that appear to arise on an environmental basis.

Three groups of investigators have reported instances in which subcutaneous sarcomas appeared in several goldfish *Carassius auratus* that had been kept in the same tank. Roffo (171) observed a small ulcerated tumor on the left side of a goldfish, just anterior to the dorsal fin. Histologically the neoplasm consisted of connective tissue arranged in bundles, among which were pleomorphic cells, some of which bore two or three nuclei. Similar tumors developed in 7 of 20 fish kept in the same aquarium. After the diseased fishes were removed and the tank was disinfected, no further tumors developed. Montpellier and Dieuzeide (138) reported that a single tumor was present in the body surface of each of five goldfish *Carassius auratus* that had been kept in the same aquarium. Histologically the lesions were identical, all were fibrosarcomas, the neoplastic fibroblasts had elongated clear nuclei with prominent nucleoli. Bits of one of the tumors were inoculated into the caudal fins of three goldfish. After the lapse of a year no tumors had developed. Six additional fish were inoculated and examined after a shorter period. They likewise failed to show tumor growth.

We ourselves (123c) have lately given an account of 30 tumor-bearing goldfish all but two of which were obtained from 3 small pools. It was found characteristic of this neoplastic disease that it is prevalent in certain pools and absent or rare in others. Where prevalent, the number of tumor-bearing fishes, as a rule, gradually increases. We have presented evidence that the factors which induce the tumors are environmental rather than hereditary. The neoplasms arise in the corium or subcutaneous tissue and are usually solitary. Their rate of growth is generally slow, although some grow rapidly and attain relatively large size (Figs. 20, 21). The histological character of these growths exemplifies the difficulty of accurately classifying mesenchymal tumors. The smaller tumors resemble fibromas or occasionally myxomas (Fig. 24), the larger fibrosarcomas (Figs. 22, 23), but the paucity of fiber-formation makes a purely fibroblastic origin doubtful. The precise histogenesis of these neoplasms is as yet undetermined. We have tentatively grouped them here with the sarcomas, although in our paper we have referred to them simply as "mesenchymal tumors." It is likely, though not as yet certain, that these tumors are similar to the goldfish tumors reported in the literature as fibrosarcomas or fibromas.

TABLE 8: SARCOMA (Unclassified)

Species	Site	Author
Minnow *Phoxinus laevis*	Corium of skin	Bugnion, 1875
Goldfish *Carassius auratus*	Dorsal fin	Bland-Sutton, 1885
Goldfish *Carassius auratus*	Not given	Semmer, 1888
Pike *Esox lucius*	Trunk muscle	Ohlmacher, 1898
Brook trout *Salvelinus fontinalis*	Trunk muscle	Plehn, 1906
Chub *Idus idus*	Trunk muscles	Plehn, 1906
Minnow *Phoxinus laevis*	Trunk muscle	Plehn, 1906
Crucian carp *Carassius carassius*	Testicle	Plehn, 1906
"LeNez" *Chondrostoma nasus*	Muscles of back	Plehn, 1906
Flounder *Psetta maeotica*	Subcutaneous (multiple)	Schroeders, 1908
Scorpion fish *Scorpaena porcus*	Subcutaneous	Schroeders, 1908
Pike *Esox lucius*	Kidney	Plehn, 1909
Danubian trout *Hucho hucho*	Liver	Plehn, 1909
Codfish *Pollachius virens*	Skin	Fiebiger, 1912
Ray *Raja macrorhynchus*	Fin ray	Drew, 1912
Eel *Anguilla vulgaris*	Mesentery	Wolff, 1912
Codfish *Gadus morhua*	Subcutaneous	Johnstone, 1912
Plaice *Pleuronectes platessa*	Subcutaneous	Drew, 1912
Codfish *Gadus morhua*	Trunk muscle	Johnstone, 1915
Croaker *Pogonias chromis*	Subcutaneous	Beatti, 1916
Conger eel *Conger conger*	Subcutaneous	Johnstone, 1920
Codfish species not given	Mouth	Johnstone, 1920
Dog salmon *Oncorhynchus keta*	Body wall	Kazama, 1922
Haddock *Melanogrammus aeglifinus*	Subcutaneous	Johnstone, 1922
Goldfish *Carassius auratus*	Subcutaneous	Schamberg & Lucké, 1922
Goldfish *Carassius auratus*	Subcutaneous	Johnstone, 1923
Codfish *Gadus morhua*	Subcutaneous	Johnstone, 1923
Ling *molva molva*	Subcutaneous	Johnstone, 1923
Turbot *Rhombus maximus*	Subcutaneous	Johnstone, 1923
Herring *Clupea harengus*	Testis	Johnstone, 1924
Eel *Anguilla vulgaris* (2)	Peritoneum	Plehn, 1924
Goldfish *Carassius auratus*	Subcutaneous	Roffo, 1924
Rainbow trout *Salmo gairdnerii* (2)	Not given	Leger, 1925
Codfish *Pollachius virens*	Subcutaneous	Johnstone, 1926
Codfish *Pollachius virens*	Mandible	Johnstone, 1926
Halibut *Hippoglossus hippoglossus*	Body wall	Johnstone, 1926
Croaker *Micropogon opercularis* (2)	Subcutaneous	Roffo, 1926
Codfish *Gadus morhua*	Subcutaneous	Thomas, 1927b
Codfish *Gadus morhua*	Ovary	Thomas, 1927b
Turbot *Rhombus maximus*	Body wall	Johnstone, 1927
Goldfish *Carassius auratus*	Subcutaneous	Dominguez, 1928
Dog salmon *Oncorhynchus keta*	Body wall	Takahashi, 1929
Dog salmon *Oncorhynchus keta*	Body wall	Takahashi, 1929
Hump-back salmon *Oncorhynchus gorbuscha*	Body wall	Takahashi, 1929
Coalfish *Theragra chalcogramma*	Body wall	Takahashi, 1929
Coalfish *Theragra chalcogramma*	Subcutaneous	Takahashi, 1929
Japanese bass *Lateolabrax japonicus*	Subcutaneous	Takahashi, 1929
Japanese bass *Lateolabrax japonicus*	Pharynx	Takahashi, 1929
Kisugo *Sillago japonica*	Base of head	Takahashi, 1929
Gurnard *Lepidoptrigla alata*	Left body wall	Takahashi, 1929
Codfish *Gadus morhua*	Body wall	Williams, 1931
Goldfish *Carassius auratus* (5)	Subcutaneous	Montpellier & Dieuzeide, 1932
Swordtail *Xiphophorus hellerii* (17)	Retrobulbar	Jahnel, 1939
Goldfish *Carassius auratus* (30)	Subcutaneous	Lucké, Schlumberger & Breedis, 1948

One of the more malignant growths was successfully transplanted to the anterior chamber of the eye of a tumor-bearing fish; all other attempts at transplantation were unsuccessful.

A possible hereditary sarcoma has been reported by Jahnel (80). A retrobulbar tumor appeared in each of four sibling tropical aquarium fish, known as swordtails, *Xiphophorus hellerii*. Seventeen other siblings were traced, 9 of which developed tumors in one or both orbits. Histologically the tumors were identical, consisting of small round or spindle-shaped cells with large vesicular nuclei; interstitial tissue was almost absent. The tumor was markedly invasive, infiltrated the orbital muscles and even penetrated the skull. Mitotic figures were numerous. In one animal a large tumor was present in the left

eye, while the right appeared grossly normal; however, on section a microscopic tumor nodule was found between the retina and choroid. The author believes that the choroid is the origin of all these tumors and that the small tumor in the right eye was primary, not metastatic. Jahnel regarded these tumors, all of which were non-pigmented, as sarcomas. It is possible however that they actually were amelanotic melanomas. In this connection see the recent papers by Levine and Gordon (111b), and by Gordon (60a).

Abstracts of other reported cases of sarcoma

BUGNION, 1875: On the left side, near the base of the tail of a minnow *Phoxinus laevis* was a pea-sized, black tumor which had appeared in a period of 2 weeks. The overlying skin was intact, though the scales were absent. The neoplasm, which was moderately soft and very vascular, contained many giant cells. The black color was only present within and immediately beneath the skin and was due to the normal pigment cells of this region. Near the tip of the caudal fin was a small black speck from which the author was able to isolate myxosporidia. The main "tumor" may likewise have been the result of such an infection, representing merely chronic inflammation with foreign body giant cells. The author identified the growth as a giant cell sarcoma.

BLAND-SUTTON, 1885: A tumor was present on the posterior border of the dorsal fin of a goldfish *Carassius auratus*. The author states that it exhibited all the histologic characteristics of a spindle cell sarcoma.

SEMMER, 1888: In a brief communication the author lists the cases of animal tumors that came to his attention throughout 23 years at the Veterinary Institute in Dorpat. He mentions a sarcoma in a goldfish, but gives no details.

OHLMACHER, 1898: Immediately beneath the dorsal fin, and embedded in the trunk musculature of a lake pike *Esox lucius*, was a spherical white tumor 5 cm. in diameter. It infiltrated the surrounding muscle and was intimately connected with the vertebrae. Lying beneath the peritoneum were 12 widely scattered tumors varying from 1 to 3 cm. in diameter. The visceral peritoneum likewise bore 24 firm white nodules 1 to 2 cm. in width; no lesions were found in the viscera. The "primary" tumor in the abdominal wall resembled small round cell sarcoma of man. It consisted of large collections of densely packed round or oval cells with prominent nuclei surrounded by scanty cytoplasm. About these collections of cells was a delicate fibrillary and richly cellular connective tissue. The metastases were similar in structure, except that the stroma was more abundant. This tumor should probably be regarded as a lymphosarcoma.

PLEHN, 1906: In the trunk musculature of a brook trout *Salvelinus fontinalis* was a large cystic tumor that contained 117 cc. of cloudy yellow fluid in which were found leukocytes, and occasional bacteria. Similar tumors in trout from the same lake had been described in 1884 by Bonnet, and later by Hofer. Though much of the tumor was necrotic, mitotic figures were common in the healthy portions. Most of the tumor cells were round and had large nuclei, but nests of spindle cells were also present. The author's diagnosis was cystosarcoma.

PLEHN, 1906: A chub *Idus idus*, 40 cm. long and 4 or 5 years old, bore a slightly bulging mass in the neck muscles, directly behind the head. On dissection, a solid white tumor the size of a pigeon egg was found. Though the center was necrotic, the periphery was actively growing and had invaded the surrounding tissue. Histologically it consisted chiefly of small round cells, though spindle cells were also present. Scattered among the tumor cells were numbers of well differentiated mucous cells.

PLEHN, 1906: Near the dorsum of the right side of a minnow *Phoxinus laevis* was a pea-sized tumor. Histologically it consisted of proliferating pleomorphic cells, the nuclei of which were very large, lobulated, and even ring-shaped. Many mitoses containing abnormal numbers of chromosomes were present.

PLEHN, 1906: A tumor arising in the testicle of a crucian carp *Carassius carassius* contained many large and small cysts. The walls were smooth and lined by flattened connective tissue; the cavities were filled with fluid or a colloidal mass. Some areas were very cellular, other regions were made up of fibrous tissue. The author identified the lesion as a cystic fibrosarcoma.

PLEHN, 1906: An egg-shaped mass, 4 x 5.5 cm., was present deep in the back muscles of "le Nez," *Chondrostoma nasus*. The tumor, which was white, spongy, and edematous, consisted of a delicate connective tissue reticulum among which were occasional multinucleate giant cells. Dilated lymphatics were numerous.

SCHROEDERS, 1908: On the pigmented surface of a flounder *Psetta maeotica* were 8 granular tumors, yellow-gray in color. Some were almost spherical, with a diameter of 5 cm., others, flat and oblong, and as much as 10 cm. in length. The masses were soft and infiltrated the adjacent skin and muscle. Histologically the parenchyma was composed of round cells of uniform size, with many mitotic figures. (Lymphosarcoma ?)

SCHROEDERS, 1908: A spindle cell sarcoma in the tail muscles of a scorpion fish *Scorpaena porcus* grew rapidly for two months when the fish was kept in an aquarium. Cachexia became marked and the animal died. Near the anal fin was an ulcerated area, 3 cm. in diameter; on section the adjacent muscles were found to be extensively infiltrated by the firm white tumor. In the liver was a white nodule the size of a pea that protruded above the surface and infiltrated the liver tissue. Under the epicardium of the heart were 6 white nodules, each the size of a pin-head. Two larger firm nodules were found in the wall of the auricle, occupying its entire thickness. Histologically the nodules in the liver and heart were similar to the primary lesion which consisted of bundles of connective tissue that were

growing between muscle fibers and had eroded the underlying bone.

PLEHN, 1909 and 1924: A large tumor of the kidney was found in a 10 pound pike *Esox lucius*. It had mestastasized to the trunk muscles. Histologic studies were of little value due to poor fixation of the tissue, but the author regarded the tumor as a sarcoma.

PLEHN, 1909: On the surface of the liver of a large Danubian trout *Hucho hucho* were white nodules varying from the size of a millet seed to that of half a walnut. On section they were found not to be circumscribed, but had invaded the normal tissues. The author identified the lesion as a spindle cell sarcoma.

FIEBIGER, 1912: According to the author this is the first record of a fibrosarcoma in a salt water fish, the three previous cases were found in fresh water fishes. A cauliflower-like mass the size of an egg grew in the subcutaneous tissue of a codfish *Pollachius virens*. It was not sharply demarcated from the adjacent tissue; the overlying skin was ulcerated, and hemorrhages were numerous on the surface of its warty excrescences. The round, elongated, and even branched tumor cells infiltrated the muscle.

DREW, 1912: A circular tumor, 4 x 1¼ inches, was present on the dorsal surface near the lateral fin of a ray *Raja macrorhynchus*. It was a broadly sessile, cauliflower-like mass covered by a layer of epithelium. The parenchyma consisted of fibrous tissue apparently derived from the perichondrium of one of the fin rays. Nowhere was the tissue very cellular, but because of its mode of growth the author classified it as a sarcoma.

WOLFF, 1912: A tumor the size of a man's fist lay between the layers of the mesentery of a large eel *Anguilla vulgaris*. Histologically, nests of tumor cells surrounded the blood vessels and were separated from one another by bands of connective tissue. The author identified the growth as a fibrosarcoma.

JOHNSTONE, 1912: On the snout of a codfish *Gadus morhua* was a gray-black non-encapsulated tumor, 7.5 x 4 x 3 cm. The superficial epidermis was destroyed. Grossly it appeared to be a typical fibroma, but histologic examination showed evidence of infiltration and increased cellularity.

DREW, 1912: A plaice *Pleuronectes platessa* bore a white ovoid mass, three-fourths of an inch in diameter, on the operculum of the pigmented side. Histologically it was made up of connective tissue. No further details were given, and the growth was listed as sarcoma by the author.

JOHNSTONE, 1915: On the right side of an emaciated codfish *Gadus morhua* between the first ventral and second dorsal fin was an 8 cm. tumor that had invaded the adjacent muscle fibers and undergone central liquefaction necrosis. The tumor cells were spindle shaped; occasional giant cells were present.

BEATTI, 1916: Numerous subcutaneous nodules were present on each operculum, on the snout, on the body, and on one side of the tongue of a croaker *Pogonias chromis*. The parenchyma consisted of densely grouped spindle cells with abundant cytoplasm and small nuclei.

JOHNSTONE, 1920: On the top and left side of the head of a conger eel *Conger conger* were two firm, spherical tumors, each 10 cm. in diameter. Histologically there was no evidence of encapsulation; the parenchyma presented all the characteristics of a mixed cell sarcoma.

JOHNSTONE, 1920: Several tumors were present about the left orbit and within the mouth of a codfish. On the basis of their histologic structure the author classified them all as sarcomas of mixed cell type.

KAZAMA, 1922: Among 1,000 salmon *Oncorhynchus keta* the author found a single tumor-bearing animal. In a five year old fish, 85 cm. in length, there was a tumor, 6.5 x 6 x 3 cm., which arose from the body wall near the origin of the right pelvic fin. It was hemispherical, gray-white, and encroached on the peritoneal cavity. The tumor was composed of fusiform cells that destroyed and infiltrated the surrounding tissues; no mitoses were seen.

JOHNSTONE, 1922: On the top of the head of a haddock *Melanogrammus aeglifinus* was a tumor which the author called a sarcoma. No further details were given.

SCHAMBERG and LUCKÉ, 1922: A goldfish that had lived in a garden pool for 15 years bore three tumors on its right side. The growth which the authors regarded as primary was on the back just anterior to the dorsal fin; it measured 2.6 cm. in diameter and projected 1.5 cm. above the skin surface. The other tumors were located near the tail, one measured 1 cm. in diameter, the other 3 mm. All were dull white in color. Infiltration of the underlying muscle had occurred about the largest tumor. Histologically the growths were composed of loosely arranged spindle cells; the blood supply was scanty; the vessels very thin walled. The two smaller tumors did not infiltrate; the authors believed they represented metastases; this interpretation is now regarded as probably erroneous.

JOHNSTONE, 1923: At the base of the tail of a goldfish *Carassius auratus* was a tumor that measured 1 x 1.5 cm. Histologically it was made up of proliferating connective tissue that had invaded the surrounding muscle.

JOHNSTONE, 1923: A mass 6 x 3 x 3 cm. was located on the anterior border of the right orbit of a codfish *Gadus morhua*. The connective tissue was abundant. but in some areas there were large numbers of small round cells, the neoplasm infiltrated the dermis and muscle, but the skin was not ulcerated.

JOHNSTONE, 1923: On the right side of the head of a ling *Molva molva*, arising above and behind the orbit, was a tumor 11 x 5 cm. which grew forward over the eye and almost covered the pupil. No histologic details were given; it was classified by the author as a fibrosarcoma.

JOHNSTONE, 1923: On the pigmented side of a turbot *Rhombus maximus* was a tumor 8 x 3 cm. Histologically the lesion consisted of connective tissue among which were spindle cells. There was little evidence of malignancy, although the author diagnosed it a sarcoma.

JOHNSTONE, 1924: A creamy white tumor, slightly nodular and measuring 3½ x 1½ inches, was loosely

attached to the parietal peritoneum of a herring *Clupea harengus*. It was fused with testis and was composed of vesicular neoplastic cells. (Lymphosarcoma ?)

PLEHN, 1924: In each of two eels *Anguilla vulgaris* there was a firm connective tissue tumor that arose in the peritoneum and greatly distended the abdomen. The author gives no details but considers the tumors to be fibrosarcomas.

LEGER, 1925: In discussing thyroid tumors in trout the author mentions 2 fibrosarcomas in rainbow trout *Salmo gairdnerii*. They were red in color and very vascular. No further details were given.

JOHNSTONE, 1926: On the top of the head of an emaciated codfish *Pollachius virens* was a tumor that measured 2 inches in diameter. On section the lesion was soft and necrotic. No histologic studies were made, but the lesion was listed by the author as a sarcoma.

JOHNSTONE, 1926: A codfish *Pollachius virens* bore a spherical tumor 8 cm. in diameter, on the anterior median aspect of the mandible. The tumor extended into the soft tissues of the mouth. Histologically it consisted of neoplastic connective tissue cells.

JOHNSTONE, 1926: A superficial ulcer, measuring 8 x 5 inches and with raised edges, was observed on the trunk surface of a halibut *Hippoglossus hippoglossus*. The lesion was an ulcerated tumor that had deeply infiltrated the underlying muscle. Microscopic examination revealed that it was composed of loosely arranged spindle cells.

ROFFO, 1926: Each of two croakers *Micropogon opercularis* bore a tumor. In one a nodular ulcerated mass 2 cm. in diameter was found at the junction of the head and thorax; in the other a similar mass was present at the base of the tail. Histologically each was a spindle cell sarcoma.

THOMAS, 1927 b: A subcutaneous tumor the size of a hen's egg was found in the trunk of each of 3 codfish *Gadus morhua*. Several areas of necrosis were present; the cells were large and fusiform, arranged in bands and whorls that resembled the uterine fibroleiomyoma of man. The author regarded the tumor as a fibrosarcoma.

THOMAS, 1927 b: In a codfish *Gadus morhua* a tumor the size of an almond was present near the right ovary. The cells are fusiform; confluent vascular spaces lined by a rudimentary endothelium were abundant. The tumor was likened by the author to the fibrosarcomas of birds and mammals.

JOHNSTONE, 1927: Multiple small round cell sarcomas were found in a turbot *Rhombus maximus*. Two of the tumors were the size of a hen's egg and were located on the pigmented side of the fish; nine additional nodules were found scattered throughout the musculature. None of the tumors were encapsulated; the adjacent muscle was infiltrated. The cells were of the small round cell type and appeared to be malignant. The author believes that the numerous tumors were multicentric in origin rather than metastatic. (Lymphosarcoma ?)

DOMINGUEZ, 1928: A 25 cm. goldfish *Carassius auratus* was observed to have had a nodule the size of a pea on its dorsal fin for 3 years. During the last year and a half the tumor had grown to a diameter of 1.5 cm. Several days before death a few "secondary" nodules were observed on the caudal fin. At autopsy, several other tumors regarded as metastatic were found in the liver and elsewhere. The primary tumor was very vascular and soft. Histologically it consisted of elongated fusiform cells with large oval nuclei.

TAKAHASHI, 1929: A tumor the size of a fist arose in the trunk musculature of a dog salmon *Oncorhynchus keta*. It was fluctuant, and was found to contain a central area of liquefaction necrosis. Histologically the tumor was composed of spindle cells that showed active mitosis and infiltrated the surrounding musculature.

TAKAHASHI, 1929: On the left side of a dog salmon *Oncorhynchus keta*, 15 cm. anterior to the tail fin, was a tumor 9 x 7 cm., lying athwart the lateral line. On the opposite side was a similar but smaller tumor. Both were soft in consistency, showed liquefaction necrosis, and infiltrated the surrounding muscle. The cells varied in size and shape and were rich in cytoplasm.

TAKAHASHI, 1929: Scattered over the body surface of a hump-back salmon *Oncorhynchus gorbuscha* were 9 tumor nodules ranging from 0.5 to 3 cm. in diameter. There was no sharp demarcation from the surrounding tissue and on histologic grounds the author identified the tumor, which was very cellular and contained many mitoses, as a round cell sarcoma. (Lymphosarcoma ?)

TAKAHASHI, 1929: In the middle of the trunk, above the lateral line of a coalfish *Theragra chalcogramma* was a tumor 4 x 3.5 cm. It was uniformly firm, and gray-white. Microscopically the parenchyma consisted of spindle cells, many of which were in mitosis. The surrounding tissue was infiltrated by the tumor.

TAKAHASHI, 1929: An oval, subcutaneous tumor larger than a hen's egg was found in the occipital region of a coalfish *Theragra chalcogramma*. Except at its base, the tumor was well demarcated from the surrounding tissue; mitotic figures were uncommon. The tumor was identified by the author as a spindle cell sarcoma.

TAKAHASHI, 1929: Eight tumors of varying sizes were present in the subcutaneous tissue of a Japanese bass *Lateolabrax japonicus*. The author identified the lesions as spindle cell sarcomas that were infiltrating the surrounding tissue.

TAKAHASHI, 1929: In the pharynx of a Japanese bass *Lateolabrax japonicus* was a tumor, 7.5 x 3 cm. Two similar lesions were present in the subcutaneous tissue of the right side. Histologically the parenchyma consisted of spindle cells with few mitotic figures.

TAKAHASHI, 1929: On the dorsum of the neck region of a kisugo *Sillago japonica* was a hemispherical, soft elevation that measured 2.2 x 1.3 cm. and was gray-white on section. It had infiltrated the adjacent tissues and was identified by the author as a spindle cell sarcoma.

TAKAHASHI, 1929: A vascular, gray, ulcerated tumor the size of a walnut was present in the trunk musculature of a gurnard *Lepidoptrigla alata*. The tumor cells were large and round with rather small nuclei. The

author regarded the tumor as a large round cell sarcoma. (Lymphosarcoma ?)

WILLIAMS, 1931: An egg-shaped tumor, 12.8 x 8.8 cm., was present in the trunk muscles of a codfish *Gadus morhua*. Histologically it consisted of small round cells embedded in a connective tissue stroma. The tumor was vascular and infiltrated the adjacent muscle. (Lymphosarcoma ?)

MYXOMA

Although in mammals fully developed mucous tissue is found only in the umbilical cord, it has been described as occurring in certain areas of some fishes, particularly about the cranial cartilage of selachians. Six cases of myxoma have been reported; these were found in 5 species of fishes, all teleosts (Table 9). Of these, four have been identified by the authors as myxofibromas, the remaining two were classified as myxosarcomas because of their invasive character.

The benign tumors arose in the subcutaneous tissue; in one case they were multiple, and grouped about the base of the fins. One of the invasive tumors was retro-orbital, the other lay within the trunk musculature; no metastases were found.

Abstracts of reported cases of Myxoma

PLEHN, 1906: The right eye of a 6 or 7 year old tench *Tinca vulgaris* was displaced by a tumor arising below the orbit. It was hemorrhagic, not sharply demarcated, and infiltrated the surrounding tissues. Histologically, some areas consisted of dense masses of spindle cells; in other regions these were separated by a mucoid ground substance in which were many delicate fibrils.

SCHROEDERS, 1908: On the pigmented side of a flounder *Psetta maeotica* was a spherical pedunculated tumor 9 x 12 x 7 cm. The overlying skin was intact. The mass was elastic in consistency and translucent on section, with numerous transecting fibrous bundles. Microscopically the large spaces between the interlacing bundles of connective tissue were filled with an amorphous substance (mucin) that stained blue with hematoxylin. Within this were scattered stellate cells. Large blood vessels coursed along the fibrous trabeculae.

SCHROEDERS, 1908: About 10 tumors were present at the junction of the trunk and fins of a flounder *Psetta maeotica*. Most were broadly sessile; in a few the overlying skin was ulcerated. Elastic in consistency, the tumors on section were translucent and yellow-white. Histologically, interlacing bundles of connective tissue, mostly arranged perpendicular to the skin, formed a coarse network, in the meshes of which was an amorphous ground substance containing stellate cells.

MACINTOSH, 1908: A plaice *Pleuronectes platessa* bore an elongated swelling on its right side. On dissection the tumor was gelatinous in consistency; the surrounding muscle was atrophied and the growth could be removed with ease. Histologically it was a loose, areolar connective tissue, enmeshed in which were many small cells and capillaries. The surrounding structures were not infiltrated.

JOHNSTONE, 1926: A tumor 2 inches in diameter, irregular in shape, and glistening white in color, was present in the body cavity of a sole *Solea solea*. Histologically the growth consisted of interlacing bundles of connective tissue and areas of areolar tissue containing stellate cells separated by large interspaces.

WILLIAMS, 1929: A tumor, 12.7 x 5.1 cm., was found in the trunk musculature on the non pigmented side of a turbot *Rhombus maximus*. The overlying skin was ulcerated. Histologically typical myxomatous tissue infiltrated the adjacent muscle.

CHONDROMA

Although the entire skeleton of selachians is composed of cartilage and this tissue is also abundant in the teleost fishes, only 8 reports of tumors of cartilage can be found in the literature (Table 10). Of these 2 were in selachians; one arose from the fin ray of a cat shark, the other from the lumbar vertebrae of a dogfish. An explanation for this dearth of material may be found in the fact that the selachians are not commonly used for food and are therefore seldom examined; however, the dogfish is frequently dissected in biology laboratories. Mulsow (140) reported a chondroma attached to the cranial bones in each of three crucian carp *Carassius carassius*. All the tumors were massive and nearly filled the cranial cavity. Unfortunately, no further data are available, but if the animals were siblings, the possibility that the tumors were comparable to multiple hereditary chondromas of man would need to be considered. There is no record in the literature of a malignant cartilagenous neoplasm of fishes.

Abstracts of reported cases of chondroma

MULSOW, 1915: A chondroma was found attached to the cranial bones in each of three crucian carp *Carassius*

TABLE 9: MYXOMA

Species	Site	Author
Tench *Tinca vulgaris*	Subcutaneous	Plehn, 1906
Flounder *Psetta maeotica*	Skin, head	Schroeders, 1908
Flounder *Psetta maeotica*	Skin, trunk & fins	Schroeders, 1908
Plaice *Pleuronectes platessa*	Body wall	MacIntosh, 1908
Sole *Solea solea*	Peritoneum	Johnstone, 1926
Turbot *Rhombus maximus*	Trunk musculature	Williams, 1929

TABLE 10: CHONDROMA

Species	Site	Author
Crucian carp *Carassius carassius* (3)	Skull bones	Mulsow, 1915
Barbel *Barbus fluviatialis*	Mandible	Surbeck, 1917
Sea pike *Elops saurus*	Subcutaneous	Surbeck, 1921
Minnow *Phoxinus*	Mandible	André, 1927
Dogfish *Squalus mitsukurii*	Lumbar vertebrae	Takahashi, 1929
Codfish *Pollachius virens*	Pre-orbital	Thomas, 1932d
Cat shark *Scylliorhinus canicula*	Skin	Thomas, 1933b
Jewel fish *Hemichromis bimaculatus*	Operculum	Nigrelli & Gordon, 1946

carassius. The tumors were massive and nearly filled the cranial cavity. These cases were cited by Thomas (1932 b) and illustrated by Plehn, 1924.

SURBECK, 1917: In a very lean barbel *Barbus fluviatialis* a tumor was found in the posterior region of the mandible. Approximately 1.5 cm. in diameter, it was formed by the apposition of 2 spherical masses. The skin over the anterior nodule was ulcerated, the mass was ossified. The posterior nodule was composed entirely of cartilage.

SURBECK, 1921: A sea pike *Elops saurus*, 100 cm. in length, bore a pedunculated tumor the size of 2 fists on its ventral surface behind the pectoral fins. It consisted of dense plaques of fibrous tissue and cartilage.

ANDRÉ, 1927: A minnow *Phoxinus* had a pedunculated mulberry-like mass attached to the tip of the mandible. The overlying skin was deeply pigmented, the head was pulled down by the weight of the tumor. Histologically, the tumor consisted of hyaline cartilage.

TAKAHASHI, 1929: A tumor, 10 x 7 x 5 cm., arose in the region of the third to sixth lumbar vertebrae of a dogfish *Squalus mitsukurii*. The overlying skin was ulcerated. The tumor, which consisted of hyaline cartilage, was fused with the third lumbar vertebra and showed a central area of liquefaction necrosis the size of a walnut.

THOMAS, 1932 d: Two symmetrical pre-orbital tumors, each measuring 1.5 x 3 cm., were found in a 6 year old codfish *Pollachius virens*. Covered by intact skin, they were broadly sessile upon the lachrymal bones, but were separated from them and adjacent tissues by a fibrous capsule. The tumors were very firm, partly calcified, and consisted of typical cartilage cells embedded in a fibrous matrix.

THOMAS, 1933 b: On the right flank below the pectoral fin of a cat shark *Scylliorhinus canicula* was a tumor, the surface of which was partly denuded. It arose from the connective tissue of the corium and possessed a peripheral zone of hyaline cartilage; the central portion was calcified.

NIGRELLI and GORDON, 1946: When first observed the tumor appeared as a small swelling at the anterior margin of the right operculum of a jewel fish *Hemichromis bimaculatus*. Within two months it had attained a size of 13 x 6 x 4 mm. The total length of the fish was 52 mm. Histologically the tumor proved to be an osteochondroma consisting chiefly of hyaline cartilage arranged in a more or less irregular pattern. The fish was the only pathologic specimen in about a hundred of the same species and strain that were kept in a 150 gallon aquarium.

OSTEOMA

Osteomas are tumors frequently observed in 15 species of fishes (Table 11). The multiple osteomas in a butterfly fish reported by William Bell in 1793 is probably the first recorded account of neoplasm in a fish. The tumors in this case were the size of hazel-nuts and involved several haemal and neural spines. Since similar lesions were found in several specimens, the fish were classified as a separate species; *viz., Chaetodon arthriticus*. The tumor-bearing fish are, however, now grouped with the normal members of the species *Platax pinnatus*.

Several Japanese investigators (96, 175, 199) have described localized bony overgrowths on the ventral spinous processes (haemal spines) of the sixth to eighth caudal vertebrae of the red tai, *Pagrosomus major*. The largest series, consisting of 102 fish, was reported by Takahashi (199). The growths varied from the size of a pea to that of a large bean; rarely were they as large as a walnut. In 28 cases the tumor was single, in 64 there were 2 tumors; in 17, 3; and in 10, 4 or more. A line of fracture was visible in several of the very small tumors. Takahashi concludes that ossification of excess callus is the initial step in the formation of these tumors.

Three specimens of *Pagrosomus major* were chosen by us at random from the collection at the National Museum in Washington, and one bore tumors identical with those described by Takahashi (Fig. 25). Careful inspection of the roentgenograms (Fig. 26) and histologic sections failed to reveal convincing evidence of previous fracture. The cortex, which is very thin in the normal spinous process, was greatly thickened (Fig. 27). As the growths increased in size the cancellous bone became more prominent until only a thin cortical layer of dense bone remained (Fig. 28). The marrow spaces were filled with well vascularized adipose tissue; hematopoiesis does not occur in the bone marrow of fishes, hence its absence in the osteomas could be anticipated.

TABLE 11: OSTEOMA

Species	Site	Author
Butterfly fish *Platax pinnatus*	Neural and haemal spines	Bell, 1793
Scabbard fish *Lepidopus* sp.	Dorsal fin rays	Gervais, 1875
Butterfly fish *Platax pinnatus*	Vertebral spines	Gervais, 1875
Pike *Esox lucius*	Dental plate	Bland-Sutton, 1885
Codfish *Gadus morhua*	Superior maxilla	Bland-Sutton, 1885
Codfish *Gadus morhua*	Intervertebral disc	Bland-Sutton, 1885
Pike *Esox lucius*	Vertebra	Plehn, 1906
Flounder *Psetta maeotica*	Vertebral spines	Schroeders, 1908
Carp *Cyprinus carpio*	Skull bones	Fiebiger, 1909c
Codfish *Gadus morhua*	Vertebrae	Williamson, 1913
Croaker *Pogonias chromis*	Two vertebrae	Beatti, 1916
Red Tai *Pagrosomus major* (6)	Mandible, haemal spines	Kazama, 1924
Red Tai *Pagrosomus major* (2)	Haemal spines	Sagawa, 1925
Codfish *Gadus morhua*	Pre-maxilla	Williams, 1929
Coalfish *Theragra chalcogramma* (8)	Dorsal fin spines	Takahashi, 1929
Flatfish *Paralichthodes olivaceus*	Vertebral spine	Takahashi, 1929
Red Tai *Pagrosomus major* (102)	Haemal spines	Takahashi, 1929
Rock fish *Sebastodes inermis*	Dorsal fin	Takahashi, 1929
Speckled trout *Salvelinus fontinalis*	Fin rays	Thomas, 1932b
Cat shark *Scylliorhinus canicula*	Skin	Thomas, 1933b
Red Tai *Pagrosomus major*	Haemal spines	Lucké & Schlumberger (not published)
Angelfish *Chaetodipterus faber* (4)	First haemal spine	Lucké & Schlumberger (not published)
Angelfish *Chaetodipterus faber*	Shoulder girdle	Lucké & Schlumberger (not published)
File fish *Alutera schoepfi*	First interneural spine	Lucké & Schlumberger (not published)

In considering the etiology of these tumors the following characteristics must be taken into account: their frequent occurrence in a particular species, the predominance of the tumors in the spines of the sixth to eighth caudal vertebrae, and the predilection of the tumors for fishes over 20 cm. in length. A traumatic origin, as suggested by Takahashi, and Kazama (96) does not adequately explain all these features of the neoplasm. An elucidation of the problem must await study of the tumor in the living animal.

Chabanaud (27) has described a localized fusiform hypertrophy characteristic of certain spines of the dorsal fin in several species of fishes—*Corvina clavigera, C. nigrita,* and *Sciaenoides perarmatus.* The enlargement of the spines was absent in very young fish, but became manifest with increasing age. The author concluded that these hypertrophies were not accidental, but might be "integrated with the metabolism of the species."

Dr. L. P. Schultz, ichthyologist of the National Museum in Washington, called our attention to similar changes in the angelfish *Chaetodipterus faber.* In old individuals of this species a localized hypertrophy of the distal portion of the first interhaemal spine tends to develop (Figs. 29 and 30). The enlargement may become massive, in some instances measuring as much as 4.5 x 3 x 2.5 cm. Histologic examination revealed that the tumor consists of cancellous bone and concentrically arranged bands of more condensed osseous tissue (Fig. 31). A similar laminated structure is found in the otoliths of fishes, where it is believed to result from periodic (seasonal) fluctuations in the rate of growth. The adipose tissue that had filled the marrow cavity was still recognizable in some regions, but elsewhere had almost disappeared. This overgrowth of bone probably represents a hyperostosis, rather than an osteoma.

Reports of the sporadic occurrence of osteomas in various species of fishes are not uncommon. The tumors are usually found in the fin rays and vertebral spines, though the maxillae have been affected twice, and Fiebiger (49) reports an osteoma the size of a child's fist that arose from the frontal bone of a 900-gram carp *Cyprinus carpio.* A sagittal section of the head revealed that much of the cranial cavity had been obliterated by the growth. Histologically the tumor consisted of bone and cartilage that in its arrangement resembled normal endochondral bone formation.

The collection of fishes in the National Museum contains the bones of an angelfish *Chaetodipterus faber* found in a Florida Indian kitchen-midden. Several of the spines bear symmetrical hyperostoses, and an irregular bony overgrowth which may be classified as an osteoma is present on one of the bones (cleithrum) of the shoulder girdle (Fig. 32).

Also in the collection is the skeleton of a file fish *Alutera schoepfi* in which a large osteoma arises from the first interneural spine (Fig. 33).

Abstracts of other reported cases of Osteoma

BELL, 1793: "The skeleton of the butterfly fish *Chaetodon arthriticus* is very singular, many of the bones having tumours, which, in the first fish I saw, I supposed to be exostoses arising from disease; but on dissecting a second, found the corresponding bones had exactly the same tumours, and the fishermen informed me they were always found in this fish; and therefore conclude them to be natural to it. In Mr. Hunter's collection are two or three of these bones, but I never knew what fish they belonged to . . . These tumours are spongy, and so soft as to be easily cut with a knife; they were filled with oil." (See discussion at beginning of this section.)

GERVAIS, 1875: In a scabbard fish *Lepidopus sp.* there were numerous osteomas on the dorsal fin rays.

GERVAIS, 1875: The author points out that many hyperostoses are found in fossil as well as in living fish. In both they are most common in the vertebrae and fin rays. Gervais found a large osteoma in the fin ray of a butterfly fish *Platax pinnatus*.

BLAND-SUTTON, 1885: The author describes a bony tumor that occurred on the dental plate of a pike *Esox lucius*. Scattered through the tissue were delicate spicules of bone.

BLAND-SUTTON, 1885: From the maxilla of a codfish *Gadus morhua* there arose a heavy, bony, disc-shaped tumor, 2 inches in diameter and half an inch in thickness.

BLAND-SUTTON, 1885: An exostosis (osteochondroma?), 1 cm. in diameter, grew from the vertebrae of a codfish *Gadus morhua*. It developed from the cartilaginous intervertebral discs.

PLEHN, 1906: A stony-hard tumor the size of a walnut arose from the last vertebra of a pike *Esox lucius*. The author identified the growth as an osteoma.

SCHROEDERS, 1908: Multiple osteomas were found on the vertebral spines of a flounder *Psetta maeotica*. The tumors, 15 in number, ranged from the size of a pinhead to that of a walnut and were attached to the bony rays. All were covered by normal skin and were firm in consistency. Histologically they resembled normal bone.

WILLIAMSON, 1913: The author reports the case of a bone tumor in a codfish *Gadus morhua*. The site of origin was a vertebral body.

BEATTI, 1916: Multiple osteomas of two vertebrae in a croaker *Pogonias chromis* were described. The author states that he has seen several such lesions in this species of fish.

KAZAMA, 1924: Osteomas were found in 6 of 500 red tai *Pagrosomus major* examined. In one the tumor appeared as a nodular mass as large as a thumb-nail at the middle of the mandible. In the other 5 the tumors were multiple, developing on the ventral spinous processes at the site of union with the anal fin. The author suggests that trauma evoked the lesions.

SAGAWA, 1925: In each of 2 red tai *Pagrosomus major* a tumor the size of the tip of the little finger grew on a spinous process of one of the caudal vertebrae. Histologically, the lesion was an osteoma.

WILLIAMS, 1929: A protuberance 5 x 2 cm., grew from the anterior portion of the left premaxilla of a codfish *Gadus morhua*. The overlying skin was intact. The microscopic structure was that of typical bone, with Haversian canals and vascular spaces.

TAKAHASHI, 1929: The author reports 8 cases of exostosis (osteochondroma ?) in *Theragra chalcogramma*. In 6 fish, tumors occurred in the spines of the dorsal fin; some were as large as a small hen's egg, and one was certainly an osteoma. In the remaining 2 cases symmetrical osteomas of thumb-nail size were present near the posterior margin of the operculum.

TAKAHASHI, 1929: A spherical, firm, bony tumor occurred on the spinous process of the third lumbar vertebra of a flatfish *Paralichthodes olivaceus*. Pressure by the tumor had produced local atrophy of the adjacent vertebral body.

THOMAS, 1932 b: A 5 year old speckled trout *Salvelinus fontinalis* bore many firm, white nodules, between 3 and 7 mm. in diameter, at the base of the rays supporting the ventral portion of the caudal fin. The overlying skin was normal, except for an absence of pigment in some areas. Stages of transition between fibroblasts and osteoblasts were noted. The appearance was that of connective tissue in which a bony metaplasia was taking place.

THOMAS, 1933 b: On the back of a cat shark *Scyliorhinus cannicula*, immediately anterior to the dorsal fin, was a firm white tumor 4 x 6 mm. Much of the tumor consisted of osteoid tissue, yet in some areas osteoblasts were present and true bone was formed.

OSTEOSARCOMA

Four tumors of fishes have been reported in the literature as osteosarcomas (Table 12). One was observed in a pike *Esox lucius*, the remaining 3 occurred in codfish *Gadus morhua* and *Pollachius virens*. The series is small and the diagnoses so open to question that no conclusions can be drawn as to the general characteristics of this tumor among fishes. The tumors were all single; no metastases

TABLE 12: OSTEOSARCOMA

Species	Site	Author
Pike *Esox lucius*	Anal fin	Wahlgren, 1873
Codfish species not given	Operculum	Murray, 1908
Codfish *Pollachius virens*	Vertebra	Williams, 1929
Codfish *Gadus morhua*	Pectoral fin	Thomas, 1932b

were observed. There is no evidence of malignant transformation of one or several of the multiple osteomas noted in the preceding section.

Abstracts of reported cases of Osteosarcoma

WAHLGREN, 1873: A nodular growth 8 x 5.5 cm. was present on the anal fin of a 3-foot pike *Esox lucius*. The overlying skin was intact. On section the tumor had the appearance of bacon and was traversed by irregular bony trabeculae. Microscopically the soft tissue consisted of small, irregular cells with one or more nuclei. The cells were surrounded by partially hyalinized connective tissue.

MURRAY, 1908: In the subcutaneous tissue of the operculum of a codfish was a hemispherical bony tumor measuring 2 cms. Histologically it was composed of irregular rounded masses of osseous tissue separated by strands of spindle cells. Thin walled, irregularly arranged capillaries were numerous.

WILLIAMS, 1929: Arising from one of the vertebrae of a codfish *Pollachius virens* was a tumor that infiltrated the adjacent muscle in all directions. Histologically it consisted of bone trabeculae and spindle cells.

THOMAS, 1932 b: Immediately behind the left pectoral fin of a young codfish *Gadus morhua* was a tumor 4.5 x 2 x 1.5 cm. that bulged into the peritoneal cavity and infiltrated the adjacent trunk muscles. The neoplasm was soft, the cut surface was gray-white and marked by many punctate hemorrhages. Among the pleomorphic cells were plaques of osteoid tissue and multinucleated giant cells.

LIPOMA

The fat storage depots of fishes correspond with those of man, though the subcutaneous tissues are not as heavily infiltrated. Nevertheless, in a catfish *Pygidium totae* found in Lake Tota, Colombia, these subcutaneous stores reach enormous proportions (Fig. 34). Dr. L. P. Schultz, ichthyologist of the National Museum, Washington, D. C., is undecided whether the adiposity is characteristic of this species, or whether it represents an abnormality. Histologically the tissue is composed of adult fat cells, the subjacent trunk musculature is not invaded, and the overlying skin is normal, without evidence of atrophy (Fig. 35). If this subcutaneous accumulation of fat is pathologic for the fish, it should probably be classified as a lipodystrophy rather than as a true lipoma.

Discrete lipomas have been reported 7 times in the literature; each tumor in a different species of fish (Table 13). All but 2 of the tumors were composed of adult fat cells. Takahashi (199) observed a lobulated lipoma, the size of a small nut, in the subcutaneous tissue behind the left eye of a Japanese samlet *Plecoglossus altivelis*. The tumor was well encapsulated, the fat cells varied in size, and the nuclei were oval or star-shaped. In 1929 Williams (228) reported a smooth spherical encapsulated tumor that had displaced the right eye of a dragonet *Callionymus lyra*. Histologically, it consisted of fat cells which the author believed had undergone malignant transformation. The connective tissue supporting stroma was myxomatous.

Abstracts of other reported cases of Lipoma

BERGMAN, 1921: On the trunk of a lake pike *Esox lucius* there was a subcutaneous tumor the size of a man's fist. Histologically the lesion was a lipoma.

BERGMAN, 1921: Beneath the skin of the pigmented surface of a plaice *Pleuronectes platessa* was a tumor that measured 6.5 x 3.5 x 3 cm. It was easily separated from the surrounding tissue and had produced a groove in the back musculature by pressure atrophy. It weighed 25 gm., was firm but elastic, and floated in water; its parenchyma was composed of adult fat cells.

KAZAMA, 1924: Within the ventral trunk musculature of a flounder *Paralichthys olivaceus* was a tumor about the size and shape of a little finger. It was encapsulated, soft in consistency, and made up of fat cells.

WILLIAMS, 1929: Protruding into the posterior portion of the body cavity of a halibut *Hippoglossus hippoglossus* was a tumor that measured 27.6 x 12.5 cm. It arose between the muscle bundles of the trunk. The cut surface appeared oily, was pale yellow, and had the consistency of cheese. Histologically the tumor was composed of well differentiated fat cells.

THOMAS, 1933 d: A coalfish *Pollachius carbonarius*, approximately 7 years old, bore a tumor, 3 x 2.5 x 2 cm., on the lower border of the liver. The surface was smooth and greenish, and covered by normal peritoneum. On section it was soft in consistency, homogeneous and without apparent fibrous trabeculae. The tumor consisted of adult fat cells, among which were islands of hematopoietic tissue.

LEIOMYOMA

The four cases of smooth muscle tumors reported

TABLE 13: LIPOMA

Species	Site	Author
Plaice *Pleuronectes platessa*	Subcutaneous	Bergman, 1921
Pike *Esox lucius*	Subcutaneous on trunk	Bergman, 1921
Flatfish *Paralicthys olivaceus*	Trunk muscle	Kazama, 1924
Halibut *Hippoglossus hippoglossus*	Trunk muscle	Williams, 1929
Dragonet *Callionymus lyra*	Subcutaneous on head	Williams, 1929
Jap. samlet *Plecoglossus altivelis*	Subcutaneous	Takahashi, 1929
Coalfish *Pollachius carbonarius*	Liver	Thomas, 1933d

in fishes (Table 14) are not convincingly neoplastic in character. In the three instances involving the stomach the lesions were diffuse and more suggestive of hyperplasia than of true tumor; one was associated with a parasitic infestation of the pyloric appendages. Plehn (157) reported a leiomyoma in a shiner *Alburnus lucidus* that arose in the trunk musculature, was infiltrated by lymphocytes, and contained minute hemorrhages. If the diagnosis of leiomyoma was correct, the tumor probably had its origin in the smooth muscle of blood vessels.

Abstracts of reported cases of Leiomyoma

PLEHN, 1906: On the ventral surface near the cardiac end of the stomach of an 8 or 9 year old rainbow trout *Salmo gairdnerii* was a bean-sized mass. The overlying mucosa was intact. Histologically the cells were identical with the normal musculature of the intestine; only the irregular arrangement was abnormal. No mitoses or inflammatory cell infiltrates were present.

PLEHN, 1906: In the trunk musculature of a shiner *Alburnus lucidus* between 1 and 2 years old, was a firm hemispherical tumor 1 cm. wide. Histologically it consisted of interlacing bundles of smooth muscle containing small "round cell" infiltrates and minute hemorrhages. Mitoses were uncommon.

PESCE, 1907: The stomach wall near the pylorus of a trout *Salmo carpio* was greatly thickened. The pyloric appendages were filled with cestodes. Histologically the cellular detail and organization differed greatly from what is regarded as normal in this region. The irregular fasciculi embedded in loose connective tissue meshes were diagnosed as fibroleiomyoma.

THOMAS, 1933 c: In a male, 3 year old herring *Clupea harengus*, 18 cm. long, the pyloric segment of the anterior gastric pouch was markedly hypertrophied. It formed a cone-shaped, white mass that was covered by peritoneum. The lumen of the stomach was normal. Histologically the mucosa and serosa were intact, the wall from two to three times its normal thickness. Oblique, regularly arranged connective tissue trabeculae formed partitions in the mass which consisted chiefly of smooth muscle fibers. There was no evidence of invasion of the surrounding structures.

RHABDOMYOMA

Rhabdomyoma has been reported in 8 species (Table 15). The record of twice as many tumors of striated muscle as of smooth muscle is probably due to the common location of rhabdomyoma in the trunk musculature where they are more readily observed. Histologically the tumors closely resemble similar tumors found in man. Noteworthy is the report by Young (235) of a rhabdomyoma in each of two turbot. The fishes had been sent from England to New Zealand and kept there for ten years in separate tanks. Tumors measuring 3½ by 1½ inches developed in both turbot at approximately the same time and in the same location on the non-pigmented side of the body. The neoplasms were soft and yellow, and histologically consisted of large striated muscle fibers, many of which were edematous and had partly disintegrated.

The only rhabdomyoma that did not arise in the trunk musculature was reported by Fiebiger (48). It was found in the viscera of a codfish *Pollachius virens* and measured 12 x 9 x 6 cm. The tumor was encapsulated and could easily be separated from the surrounding organs. Small cysts and groups of cells that resembled hepatic cells were present among the striated muscle bundles. This suggests that the growth may have been primarily a teratoid tumor in which the muscle fibers formed the predominant element.

Abstracts of other reported cases of Rhabdomyoma

ADAMI, 1908: A red trout *Salmo clarkii* bore a 3 cm., spherical tumor on its left side 4 cm. behind the dorsal fin. It was embedded in the musculature and projected 1.5 cm. above the general body surface. The tumor was well circumscribed and separated readily from the surrounding tissue. Histologically it was composed almost wholly of multinucleate giant cells. Several of these were of great length and resembled regenerating muscle. In not a few, the part of the cell farthest from the grouped nuclei showed definite striations.

BERGMAN, 1921: On the right side of a stint *Osmerus eperlanus*, directly behind the head, was a tumor, 1.5 x 1 x 1.2 cm. The gray-white mass had the consistency of normal muscle and extended to the vertebral column. Histologically it was well circumscribed by a thin fibrillar capsule. The parenchymal cells were transversely striated muscle fibers and myeloblasts in various stages of development.

KOLMER, 1928: A tumor was present in the trunk musculature of an adult tench *Tinca tinca*. The overlying scales in this region were approximately twice normal size. Microscopically the tissue consisted of irregularly arranged striated muscle fibers that formed a typical rhabdomyoma.

LADREYT, 1930: Near the posterior border of the right pectoral fin of a wrasse *Labrus mixtus* was a pea-

TABLE 14: LEIOMYOMA

Species	Site	Author
Rainbow trout *Salmo gairdnerii*	Stomach wall	Plehn, 1906
Shiner *Alburnus lucidus*	Trunk muscle	Plehn, 1906
Trout *Salmo carpio*	Stomach wall	Pesce, 1907
Herring *Clupea harengus*	Wall of stomach	Thomas, 1933c

TABLE 15: RHABDOMYOMA

Species	Site	Author
Red trout *Salmo clarkii*	Trunk muscle	Adami, 1908
Codfish *Pollachius virens*	Viscera	Fiebiger, 1909b
Stint *Osmerus eperlanus*	Trunk muscle	Bergman, 1921
Turbot *Rhombus maximus*	Trunk muscle	Young, 1925
Tench *Tinca tinca*	Trunk muscle	Kolmer, 1928
Wrasse *Labrus mixtus*	Trunk muscle	Ladreyt, 1930
Herring *Clupea harengus*	Trunk muscle	Williams, 1931
Halibut *Hippoglossus hippoglossus*	Trunk muscle	Thomas, 1932a

sized tumor. The overlying skin was ulcerated, the surrounding musculature infiltrated by the neoplasm. Histologically it consisted of fusiform and giant cells as well as myocytes. The author attempted to trace the evolution of the connective tissue cells into mature muscle fibers.

WILLIAMS, 1931: In a herring *Clupea harengus*, 24 cm. in length, a tumor 3.1 cm. in diameter arose in the dorsal musculature immediately anterior to the dorsal fin. The overlying skin was intact. Histologically only the muscle fibers in the periphery showed striations.

THOMAS, 1932 a: Along the ventral border of a halibut *Hippoglossus hippoglossus* was a tumor measuring 1.7 x 1.5 cm. On section it was pearly white and finely fasciculated. There were no metastases. Histologically the posterior portion of the tumor consisted of undifferentiated cells, but toward the anterior region the normal myomeric architecture was approached. A pseudocapsule was present, the result of pressure on the adjacent myomeres which were infiltrated by the tumor. The author discussed the embryology of fish striated muscle and recognized many of the transitional forms in this tumor.

HEMANGIOMA

Hemangiomas have been reported in 11 species (Table 16). The earliest, and to date most interesting account of such tumors is that of Plehn (157). She observed three cases in the stickleback *Gasterosteus aculeatus* and states that these growths occur frequently in this fish. The tumors, which were deep red in color, arose on the ventral surface of the head, spread forward to the angle of the mouth, and thence over the operculum to the level of the eyes. They seemed to cause little functional embarrassment. The proliferation and dilatation of the vessels began in the corium and subcutaneous tissue, then infiltrated the tissues between the gill arches and hyoid bone. Solid cords of endothelial cells finally penetrated to and partly replaced the subjacent muscle.

Johnstone has reported 7 of the 12 recorded cases of vascular tumors in fishes. He identified 5 as examples of angiosarcoma; however, histologic data in support of this diagnosis are meager. Several of the lesions reported by investigators may represent a local inflammatory response rather than a true neoplasm; Drew, for example, found a copepod within the lesion in one of his cases.

Abstracts of other reported cases of Hemangioma

MURRAY, 1908: In the pectoral girdle of a codfish *Gadus morhua* there was a flat, dark-colored growth about 1.5 cm. in diameter. Microscopically it consisted of capillaries distended with blood.

DREW, 1912: A reddish tumor was present in the midline on the ventral surface of the head of a spotted ray *Raja maculata* at the level of the fifth gill arch. Histologically the appearance was identical with that of a hemangioma of man. The body of a copepod was found within the tumor.

DREW, 1912: A nearly spherical tumor, 1 inch in diameter, was found in the region of the basi-hyal bone of a conger eel *Conger conger*. It was white and covered by an irregularly thicknened and ulcerated epidermis. The central portion of the tumor was necrotic. The neoplasm was made up of masses of endothelial cells which in some regions arranged themselves in long columns that suggested capillary formation.

DREW, 1912: A soft, red tumor was present on the

TABLE 16: HEMANGIOMA

Species	Site	Author
Stickleback *Gasterosteus aculeatus* (3)	Subcutaneous, head	Plehn, 1906
Codfish *Gadus morhua*	Pectoral girdle	Murray, 1908
Spotted ray *Raja maculata*	Subcutaneous	Drew, 1912
Conger eel *Conger conger*	Region of basi-hyal	Drew, 1912
Gurnard *Trigla lineata*	Operculum	Drew, 1912
Stickleback *Pungitius pungitius*	Iris	Johnstone, 1915
Mackerel *Scomber scombrus*	Body wall	Johnstone, 1924
Codfish species not given	Swim bladder	Johnstone, 1925
Ling *Molva molva*	Body wall	Johnstone, 1925
Plaice *Pleuronectes platessa*	Body wall	Johnstone, 1925
Codfish *Pollachius virens*	Trunk musculature	Johnstone, 1926
Hake *Merluccius merluccius*	Submucosa of rectum	Johnstone, 1926

inner surface of the operculum of a gurnard *Trigla lineata*. Histologically it consisted of a mass of dilated capillaries.

JOHNSTONE, 1915: The pupil of the left eye of a stickleback *Pungitius pungitius* was surrounded by a brown, raised, nodular swelling. The cornea was mottled with opaque specks, while small arborescent growths arose from its center. On section the tumor, consisting of a plexus of proliferating capillaries, occupied the subcutaneous subconjunctival and iridial tissues. The papillae noted on the cornea were masses of epithelial cells.

JOHNSTONE, 1924: In the body wall of a mackerel *Scomber scombrus* was a soft, red tumor, 5 x 1.5 cm., that infiltrated the adjacent musculature. It was composed of dilated capillaries supported by proliferating connective tissue.

JOHNSTONE, 1925: In the swim bladder of a codfish was a tumor that apparently arose from the plexus of vessels normally present in this region. It consisted of numerous dilated vessels supported by a loose connective tissue stroma infiltrated by collections of lymphoid tissue.

JOHNSTONE, 1925: A firm mass, 2 x 4 inches, was found in the trunk musculature of a ling *Molva molva*. A central dark area was interpreted by the author as clotted blood; the periphery consisted of many dilated vessels, capillaries, and proliferating angioblasts.

JOHNSTONE, 1925: The author reports single lesions, averaging from 2 to 3 cm. in diameter, that arose in the trunk musculature of 3 plaice *Pleuronectes platessa*. Histologically they were made up entirely of thin-walled vessels that infiltrated the surrounding tissue.

JOHNSTONE, 1926: Many hard nodules, ½ to 1 inch in diameter, were palpated throughout the trunk musculature of a codfish *Pollachius virens*. Histologically each nodule consisted of a mass of blood vessels and accompanying connective tissue stroma.

JOHNSTONE, 1926: The last 3 inches of the rectum of a hake *Merluccius merluccius* was swollen, deep red in color, and resembled a huge blood clot. On section the mucosa and submucosa were almost replaced by dilated vascular spaces, many of which contained actively proliferating angioblasts.

LYMPHOSARCOMA

A discussion of lymphosarcoma in fishes must be introduced by pointing out that circumscribed collections of lymphoid tissue in the form of nodes, do not occur in these animals. They are found in the phylogenetic scale only in some birds (aquatic) and in all mammals (93). In elasmobranch fishes, hematopoiesis takes place in the spleen, the mesonephros, the submucosa of the intestine, and the subcapsular tissue of the gonads. In teleosts the mesonephros is generally the dominant hematopoietic organ; the spleen and submucosa of the intestine have only an accessory role. This distribution of the hematopoietic tissue accounts for the frequent location of lymphosarcoma in the kidney; *i.e.*, in 15 of the 20 reported cases (Table 17).

In the older reports lymphosarcoma has not always been separated from other forms of sarcoma; it is probable that several of the tumors which in this review are included in the latter group are in fact lymphosarcomas.

Plehn (162) observed that the kidney of a goldfish *Carassius auratus* was increased to 20 times its normal size. The organ was firm and white; the normal renal tissue had been almost wholly replaced by lymphoid elements. Metastases were found in the liver. The author believes that renal insufficiency accounted for the generalized edema that was present. In a herring *Clupea harengus*, measuring 30 cm. in length, Johnstone (91) found an intracoelomic tumor, 5 x 2.5 cm. Histologically it was composed of connective tissue stroma packed with lymphoid cells. It is probable that the subcapsular tissue of the gonads was the primary site of the neoplasm. Recently, Nigrelli (142) has reported several interesting cases of metastasizing lymphosarcomas. Twelve fully grown pike *Esox lucius*, kept in one tank, succumbed at various intervals at the N. Y. Aquarium during the years 1940 and 1941. Both sexes were represented. There were no external manifestations of disease, but autopsies showed comparatively massive growths in the kidneys, with metastasis to the spleen and liver. Histologically the tumors were identified as lymphosarcomas composed mainly of large lymphoblasts supported by irregular strands of fibrous stroma and a delicate reticulum.

Another metastasizing tumor was found in a male

TABLE 17: LYMPHOSARCOMA

Species	Site	Author
Flatfish *Platichthys flesus*	Retro-orbital	Johnstone, 1912
Goldfish *Carassius auratus*	Kidney	Plehn, 1924
Herring *Clupea harengus*	Peritoneum	Johnstone, 1926
Conger eel *Conger conger*	Kidney	Williams, 1931
Amer. salmon *Salmo salar*	Kidney	Haddow & Blake, 1933
Pike *Esox lucius*	Subcutaneous	Haddow & Blake, 1933
Trop. Aquar. Fish *Rasbora lateristriata*	Peritoneum	Smith, Coates, & Strong, 1936
Pike *Esox lucius* (12)	Kidney	Nigrelli, 1947
Characin *Astyanax mexicanus*	Branchial region	Nigrelli, 1947

characin *Astyanax mexicanus* measuring 50 mm. in total length and about four years old. The growths occurred in the branchial region and had caused exophthalmia. The tumor probably originated in a paired mass of lymphoid tissue normally present in the posterior region of the branchial cavity. There was considerable local invasion extending even to the myocardium. Metastases were present in the corium of the skin, the gills, kidneys, pancreas, and liver.

Abstracts of other reported cases of Lymphosarcoma

JOHNSTONE, 1912: The left eye of a plaice *Platichthys flesus* was displaced by a retro-orbital tumor, measuring 2 x 2 cm. The growth consisted of connective tissue stroma in which were embedded masses of lymphocytes separated by large spaces, apparently lymph channels.

WILLIAMS, 1931: A very large bulge was seen in the ventral wall of a conger eel *Conger conger*; the overlying skin was intact. Dissection disclosed a retroperitoneal tumor, 16 x 9 cm., in the region of the kidney. Within the kidney the lymphoid tissue was greatly increased and the renal tubules were widely separated; mitoses were numerous.

HADDOW and BLAKE, 1933: In each kidney of a 5 year old American salmon *Salmo salar* there was a mass, 4.4 x 2.4 cm., that diffusely invaded these organs, resulting in almost complete destruction of the renal units. The haemal arch was occluded by a mass of tumor tissue, apparently by direct extension from the veins of the renal portal system.

HADDOW and BLAKE, 1933: Many subcutaneous tumors were found about the bases of the fins and within the buccal cavity of a partly decomposed pike *Esox lucius*. The neoplastic tissue was gray in color and did not infiltrate the surrounding musculature; visceral metastases were absent. Grossly the lesions appeared mucoid or fatty in character; histologically they appeared to be lymphoid in origin. The authors suggest lymphosarcoma as a possible diagnosis.

SMITH, COATES and STRONG, 1936: Neoplastic lymphoid tissue produced a mass that was first noted in the upper abdomen near the liver in a tropical aquarium fish *Rasbora lateristriata*. The intra-abdominal swelling could be seen distinctly during life through the semitransparent abdominal wall. After approximately 2 months the tumor had extended cephalad, involving the gill on the right side and pressing the operculum outward.

TUMORS OF PIGMENT CELLS

Fishes are provided with an abundance of pigment cells, not only in the epidermis and subcutaneous tissues, but in the peritoneum, mesentery, viscera, and central nervous system. The most common of these cells in fishes as in other vertebrates are the melanophores which elaborate melanin. The guanophores, laden with guanin crystals, and the iridiocytes, bearing crystals of inorganic salts, are responsible for the white background and the iridescence so characteristic of fish coloration. In addition, most fishes also possess yellow-pigmented xanthophores and red-pigmented erythrophores (allophores).

The close relationship of these variously pigmented cells has been generally accepted by investigators, and their origin usually sought in a common undifferentiated mesenchymal cell (8). In 1909 Borcca suggested that these cells may be derived from the neural crest. In recent years investigators working with amphibian and avian embryos have accumulated convincing evidence that in these animals the neural crest does supply the pigment cells (42). In the light of these findings it is probable that the pigment cells of fishes have their origin in neurectoderm.

MELANOMA

The most frequently observed pigment cell tumors of fishes are the melanomas located in the skin and subcutaneous tissues. They have been reported in 12 species (Table 18). Whether they are predominantly benign or malignant cannot be clearly established. Johnstone (82, 83, 85) and Prince (165) reported cases in which the lesions were multiple, but whether they represent metastases is difficult to determine. Besides these sporadic examples, melanotic tumors are commonly found in hybrids of the tropical aquarium fishes *Xiphophorus hellerii* and *Platypoecilus maculatus*. First reported by Haüssler (1928) and Kosswig (1929) they have been thoroughly investigated by Gordon and his coworkers (59 to 63, 169). Swordtails *Xiphophorus hellerii* possess only micromelanophores, or no melanophores at all; platyfish *Platypoecilus maculatus* bear both micro- and macromelanophores. It is the platyfish macromelanophore factor introduced into the hybrid that produces melanosis and melanotic neoplasms. Although the dominant inherited factor responsible for melanosis is that for macromelanophores of the platyfish, alone it is ineffectual. The swordtail strain also plays its part in the production of the lesion, since it contributes hereditary factors that initiate the multiplication of the macromelanophores in the hybrids.

According to the descriptions of Reed and Gordon (169), the neoplastic disease begins as a densely pigmented spot in the caudal fin. It increases in size and merges with other pigmented regions until a large area is involved. This stage has been observed in day-old fish as well as in embryos 6 days before hatching (59). Subsequently a local swell-

TABLE 18: MELANOMA

Species	Site	Author
Haddock *Melanogrammus aeglifinus*	Skin	Prince, 1892
Thorn-back ray *Raja clavata*	Skin of head	Johnstone, 1911
Thorn-back ray *Raja clavata*	Skin of head	Johnstone, 1912
Ray *Raja batis* (2)	Skin of fins	Johnstone, 1912
Ray *Raja batis* (2)	Skin of fins	Johnstone, 1913
Halibut *Hippoglossus hippoglossus*	Skin (multiple)	Johnstone, 1915
Eel-pout *Zoarces viviparus*	Skin	Bergman, 1921
Plaice *Pleuronectes platessa*	Skin of pigmented surface	Johnstone, 1925
Killifishes (*Platypoecilus maculatus* (*Xiphophorus hellerii*	Skin	Haüssler, 1928 & 1934
Killifishes hybrids	Skin	Kosswig, 1929
Coalfishes *Theragra chalcogramma* (2)	Body wall	Takahashi, 1929
Mackerel *Pneumatophorus colias*	Skin	Takahashi, 1929
Angler fish *Lophius piscatorius*	Skin	Ingleby, 1929
Killifishes hybrids	Skin	Reed & Gordon, 1931
Thorn-back ray *Raja clavata*	Skin	Haddow & Blake, 1933
Grouper *Epinephelus gigas*	Branchial region	Dollfus, Timon-David & Mosinger, 1938
Silverside *Menidia beryllina peninsulae*	Skin	Nigrelli & Gordon, 1944
Killifishes hybrids (11)	Eye	Levine & Gordon, 1946
Killifishes hybrids (many)	Skin	Gordon, 1948

ing develops due to proliferation of the pigmented cells. The transition from melanosis to neoplasm is not abrupt, and in the early stages a distinction between the two cannot be made. It should be pointed out here that non-neoplastic melanosis is not uncommon in fishes and has recently been reported by Smith and Coates (189) in the lung fish. The neoplastic cells may be devoid of pigment, a condition frequently observed in the metastases of human melanomas; or they may be very heavily pigmented.

The growth of these melanomas in tissue culture was studied by Grand, Gordon, and Cameron (63).

Recently Levine and Gordon (111b) have reported on the occurrence of pigmented ocular tumors in xiphophorin fishes. The species distribution of the neoplasms was as follows: 1 *Platypoecilus maculatus,* 1 *Xiphophorus pygmaeus,* 6 hybrid *P. maculatus–X. hellerii* out of a brood of 15, 2 hybrids out of a brood of 16, and 1 hybrid from a private collector. All fish were full grown, at least 6 months old and some over a year old, before the tumor developed. The average life span of the normal fish is about a year and a half. Exophthalmia developed gradually and was associated with blindness of the affected eye. The neoplasms were composed chiefly of pigmented epithelioid cells. They arose in the choroid, invaded the retina, later they perforated the eyeball and grew into the retroorbital tissues. No bacterial or virus agent could be identified. Although the authors stated in their paper that the tumor is not controlled by a genetic factor, more recent work has convinced Dr. Gordon that the tumors develop on a genetic basis (60a).

These tumors closely resemble the ocular neoplasms reported by Jahnel (80) and identified by him as fibrosarcomas; these have been discussed in the section on sarcoma. He likewise sought a genetic basis for their occurrence.

A hitherto undescribed type of pigmented tumor was found in a grouper *Epinephelus gigas,* by Dollfus, Timon-David, and Mosinger. It is of such interest that we shall quote an abstract made by Foulds (35a) in full. "The primary growth was almost certainly of branchial origin. In addition to tumors in the branchial region there were smaller subcutaneous tumors in the caudal, anal, and dorsal fins and in the liver and spleen. Some of the tumors, especially in the branchial region, were plainly epithelial, resembling basal cell tumors of mammals. Some of the cells were pigmented and, since pigmentation seemed associated with an eosinophilic degeneration, it appeared as if the melanin granules represented a degenerative phenomenon. In the liver and spleen, however, though almost all the tumor cells were pigmented there was no sign of a degenerative process; careful examination revealed rare tumor nodules with cells like those in the branchial tumors. None of the tumor cells resembled the melanocytes characteristic of lower vertebrates. Transitions were found between epithelial cells devoid of pigment and melanotic cells in which the abundance of pigment was explicable only by an active production of melanin. The most likely hypothesis is that the tumor cells of epithelial origin acquired secondarily the power of forming melanin. Previously described melanomas in fishes were predominantly sarcomatous in appearance. It is sug-

gested that the present tumor reopens the problem of the histogenesis of melanoblastomas and that of the specificity of the melanin-forming function."

Abstracts of other reported cases of Melanoma

PRINCE, 1892: In a cachectic female haddock *Melanogrammus aeglifinus* there was a brown tumor, 20 x 5 cm., just below the lateral line. The surface was ulcerated, the surrounding skin inflamed. Three pigmented spots were present near the tumor. Histologically both pigmented and nonpigmented cells were seen; the underlying muscle was not invaded.

JOHNSTONE, 1911: On the right side of the head, behind the eye of a thorn-back ray *Raja clavata,* was a black, elliptical tumor, 7.5 x 6 x 2 cm. The animal was so emaciated that the skeleton was visible. The skin over the neoplasm was intact, though infiltrated by tumor cells. Histologically the growth was made up of irregular interlacing bundles of spindle cells that were heavily laden with pigment and had infiltrated the surrounding tissues. No metastases were found.

JOHNSTONE, 1912: A thorn-back ray *Raja clavata,* 2 feet in length, bore a spherical tumor 7 cm. in diameter. The growth, which was located on the dorsum of the head at eye level, was black and very soft. The pigmented tumor cells were short spindles that infiltrated the adjacent tissues. At the posterior margin of one eye was a firm white tumor apparently a fibroma.

JOHNSTONE, 1912: Melanotic tumors were found in the skin of two rays *Raja batis.* One of the fish was caught off the coast of Ireland, the other near Scotland. In one the growth was found on the pectoral fin; it was small and was not examined histologically. In the other fish a flat black tumor, 10 x 6 x 1 cm., was present near the center of the dorsal surface of the left pectoral fin. Several 1 cm. nodules were scattered elsewhere on the fin surface; all were covered by normal epithelium. The tumor cells were pleomorphic, rich in melanin granules, and infiltrated the adjacent tissue.

JOHNSTONE, 1913: Melanotic sarcomas were found in two rays *Raja batis.* In one a flat pigmented lesion rose a millimeter or more above the surface of a pectoral fin. In the other, caught off the coast of Ireland at a depth of 70 fathoms, and measuring 5 ft. in width, there were many melanotic tumors scattered over the surface. The largest was on the back, measured 15 x 7 x 2.5 cm., and was very soft. On section, the densely pigmented spindle cells deeply invaded the adjacent muscle.

JOHNSTONE, 1915: Scattered over the body surface of a halibut *Hippoglossus hippoglossus* were numerous tumor nodules. Histologically, they consisted of spindle cells, some multinucleate, others containing melanin granules. The tumor cells infiltrated the surrounding muscle tissue.

BERGMAN, 1921: On the right side of the tail of an eel-pout *Zoarces viviparus* was a black tumor the size of a hazel nut. Cross sections of the body revealed strands of black tissue extending to the vertebrae and diffusely infiltrating the muscle. Most of the cells were spindle shaped with a homogeneous cytoplasm that occasionally contained a few pigment granules. Other cells were branched, rich in pigment, and not distinguishable from melanophores. The author believed that the nonpigmented cells were melanophores that had developed too quickly for the pigment to form.

JOHNSTONE, 1925: Behind the pectoral fin on the pigmented side of a plaice *Pleuronectes platessa* was a circular tumor 3 x 0.5 cm. with raised edges and a mammillated surface, which was variegated black and orange. The corium was increased in width by the tumor cells, which resembled short thick connective tissue cells; the masses of pigment were mostly extracellular. The surrounding muscle was infiltrated.

HAÜSSLER, 1928: Melanomas, derived from melanophores in hybrids of the swordtail *Xiphophorus hellerii* and platyfish *Platypoecilus maculatus,* Mexican killifishes, were large and infiltrative, though no metastases were noted. The author reports 8 cases, involving 20 of the F_2 generation; there was one case in a male F_1. All tumors were located near the midline on the ventral surface.

KOSSWIG, 1929: Melanotic tumors were observed in hybrids of female *Xiphophorus hellerii* and male *Platypoecilus maculatus.* In F_1, the hybrids were much blacker than the male *Platypoecilus* because the black factor—Gene N—acts differently on the female *Xiphophorus* than on the female *Platypoecilus.* By again breeding a dark hybrid with *Platypoecilus,* the pigmentation became even more pronounced, and the proliferation of the melanophores formed small melanomas on the surface; they also infiltrated the internal organs.

TAKAHASHI, 1929: In the middle of the left side of the body near the lateral line of a coalfish *Theragra chalcogramma* was a tumor that measured 9 x 5 x 5 cm. It was mottled gray and black, rather soft in consistency and sharply demarcated, except at its base. The tumor cells were round, spindle, or starshaped, embedded in an abundant vascular connective tissue stroma. On the left side of another coalfish, more emaciated than the last, was a soft, uniformly black tumor, infiltrating the skeletal muscle. A subperitoneal, black, pea-sized nodule of the same histologic structure was probably a metastasis. The cells were oval, many contained pigment granules.

TAKAHASHI, 1929: On the left side of a chub mackerel *Pneumatophorus colias* was a tumor, 3 x 4 x 1.5 cm., which on section was yellow-brown in color. The tumor parenchyma consisted of small round or oval cells some of which contained small amounts of melanin. A few large pigment-bearing cells were scattered among them. The vascular tumor had a scanty stroma, and actively infiltrated the surrounding tissue.

INGLEBY, 1929: A black warty tumor, apparently arising in the subcutaneous tissue, was found in a northern angler fish *Lophius piscatorius.* The main mass of the tumor lay above the level of the skin and was not encapsulated. Histologically it consisted of large branched cells loaded with masses of pigment which in most instances completely obscured the nuclei. The

author suggested that the tumor represented a homologue of the pigmented mole in man.

HADDOW and BLAKE, 1933: The authors report two skin tumors in a thornback ray *Raja clavata*. Both neoplasms were sessile and lobulated. The larger measured 4 x 2.1 cm., was distinctly paler than the normal skin, but contained scattered deposits of melanin; the smaller, measuring 2 x 1.5 x 0.5 cm., was heavily laden with melanin. Histologically they were richly cellular, composed of dense collections of melanoblasts possessing extremely large nuclei. Each tumor was traversed by coarse fibrous trabeculae and rifted with many thin-walled blood sinuses. The melanin was present mainly in the form of intracellular granules, though some was found in the tissue spaces.

HAÜSSLER, 1934: A second report by this author about melanomas that occurred in hybrids of *Xiphophorus* and *Platypoecilus*. In the 5 cases presented the tumors were found in the region of the tail and were derived from macromelanophores. Whether the tumors were benign or malignant was not established.

NIGRELLI and GORDON, 1944: A melanotic tumor was found in a silverside *Menidia beryllina peninsulae*. The fish measured 4.7 cm. in length; the tumor covered an area of 5 x 1.5 x 1.0 mm. and extended along the sides of the body. In the normal fish a broad band of melanophores forms a syncytium in the region of the lateral line; these may have been the cells of origin of the tumor. Histologically, the pigment cell overgrowth in this fish appeared to be quite like the melanotic tumors reported in hybrid killifishes by Reed and Gordon. The tumor contained melanin-bearing cells of different sizes, corresponding somewhat to the variations in size normally encountered. The cells spread laterally in the corium, though in some areas they penetrated the epidermis as well as the underlying fascia, muscle, and bone.

ERYTHROPHOROMA

Tumors arising from the red pigment containing erythrophores (allophores) have been reported in a trout, a flatfish, a tunnyfish, a gurnard, and in hybrid killifish (Table 19). The most detailed account is that of Smith (188) describing the lesions in a flatfish *Pseudopleuronectes americanus*. A red tumor, 3 x 2 cm., was situated on the pigmented side 4 cm. behind the right operculum, where it invaded the subjacent tissues to the depth of the extraperitoneal muscles. Similar though smaller lesions were scattered over the body: 2 near the posterior margin of the main mass, and 2 on the nonpigmented side. Visceral tumors were also present: 10 in the liver, 10 in the kidneys, and 3 in the spleen where they nearly replaced the normal parenchyma. Microscopically masses of spindle-shaped cells supported by delicate capillaries extended throughout the thickened corium as far as the underlying muscle bundles. Frozen sections of fresh tumor tissue showed small pigment granules, varying in color from vermilion to orange, and present in many of the cells. Smith regarded the large skin lesion as the primary site of the tumor and the smaller nodules as metastases.

In 1929 Kosswig reported erythrophoromas in two male fish produced by back-crossing an F_1 red finned black killifish hybrid to a pure line red-finned platyfish. In subsequent experiments (104) he obtained a similar lesion in an F_1 hybrid of *Xiphophorus montezuma* and *X. hellerii*. Kosswig interpreted the lesion as a dominant mutation in a somatic cell. Only in a single F_1 hybrid of *X. hellerii* and *Platypoecilus maculatus* did typical erythrophoroma develop. When this hybrid was crossed with a pure line *X. hellerii*, erythrophoromas were found in 5 of the offspring. Gordon (59) has suggested that the tumors described by Kosswig may represent melanomas which were invaded by erythrophores, rather than true erythrophoromas.

Abstracts of other reported cases of Erythrophoroma

TAKAHASHI, 1929: On the left side just behind the head of a gurnard *Chelidonichthys kumu* was a tumor, 8.5 x 7 x 7 cm., which infiltrated the muscles down to the vertebrae and bulged into the coelom. On section it was greenish-brown and firm. The tumor cells infiltrated the surrounding tissue, and were oval, with large vesicular nuclei and a scanty cytoplasm which in some instances contained yellow-green pigment granules. By chemical methods the cells were identified as allophores, a term accepted as synonymous with "erythrophore."

THOMAS, 1931 b: A subperitoneal erythrophoroma was found in a tunny fish *Gymnosarda alleterata*. The tumor was of the same size and color as a small orange, lay at the level of the liver, and was covered by peri-

TABLE 19: ERYTHROPHOROMA

Species	Site	Author
Gurnard *Chelidonichthys kumu*	Skin	Takahashi, 1929
Killifishes (*Platypoecilus maculatus* (9) (*Xiphophorus hellerii*	Skin	Kosswig, 1929, 1931
Tunny fish *Gymnosarda alleterata*	Peritoneum	Thomas, 1931b
Trout species not given	Skin	Thomas, 1931b
Flatfish *Pseudopleuronectes americ.*	Skin	Smith, G.N., 1934
Killifishes (*P. maculatus* (*X. hellerii*	Skin	Smith, Coates & Strong, 1936

toneum. Beneath the mesothelial cells of the peritoneum there was considerable proliferation of branched erythrophores. The protoplasmic prolongations were uniformly stippled by granules of red pigment.

THOMAS, 1931 b: An orange-red tumor infiltrated the posterior portion of the anal fin of a trout; the branching erythrophores were embedded in a dense connective tissue stroma. A small tumor, identical with that on the fin, found on the surface of the peritoneum, was interpreted by the author as a metastasis.

SMITH, COATES and STRONG, 1936: In a hybrid of *X. hellerii* and *P. maculatus* the authors found a tumor arising from the red pigment cells. Brick red in color, it grew in the region of the dorsal fin, invaded the skin and adjacent tissues but none of the deeper structures. Microscopically the pigmented tumor cells were large, round or fusiform, and bore occasional dendrites.

GUANOPHOROMA

Only a single example of a neoplasm taking origin from guanophores is on record. Takahashi (199) described a tumor, 7 x 5 x 1.5 cm., on the left side near the caudal fin of a "fat cod" *Hexagrammos atakii*. On section the tissue was silver-white and had a peculiar sheen. Histologically, it was densely infiltrated by short, yellow-white, needle shaped, doubly refractile guanine crystals of great brilliance. The crystals were present not only in the pleomorphic tumor cells but also in the interstitial tissues.

XANTHOPHOROMA

The only tumor purported to take its origin from the xanthophores in the skin of a fish was reported by Schroeders (179). An irregular orange-colored tumor, the size of a bean, was located on the right side of the trunk beneath and partly upon the dorsal fin of a blenny. In the vicinity of this tumor were several nodules the size of pin-heads. Histologically the tumor consisted principally of large epithelioid cells with vesicular nuclei and an orange-yellow pigment in the cytoplasm. Single and multinucleated, branched giant cells that also contained the pigment were scattered through the tissue. The author believed that the cells were derived from the endothelium of the lymphatic spaces and blood vessels. The center of the mass was hemorrhagic and necrotic. Whether it was a true neoplasm or a granulomatous reaction to injury must remain undecided.

TUMORS OF NERVOUS TISSUES

Only 7 papers dealing with tumors of the nervous system of 9 species of fishes were found in the literature (Table 20). There is as yet no record of a neoplasm of the brain or spinal cord: Takahashi (199) and Haddow and Blake (68) each report a ganglioneuroma arising in a spinal ganglion of a flatfish. Thomas (203) observed a similar tumor in a codfish and subsequently described a neuroepithelioma of the olfactory plate in a gilthead (210).

One of us (118) has studied the common nerve sheath tumors of the snappers *Lutianidae*. A total of 76 tumor bearing fishes were collected, which were members of three species: the gray snapper *Lutianus griseus*, the dog snapper *L. jocu*, and the schoolmaster, *L. apodus*. The neoplasms generally occurred along the course of the larger subcutaneous nerves, particularly those of the head and dorsal regions, as solitary or multiple relatively large firm white masses (Fig. 36). Histologically, they closely resembled the human neoplasms of the nerve sheath group and were composed of two kinds of tissue: one compact and richly fibrocellular (corresponding to the Antoni A type); the other loose, reticulated, and poorly cellular (Antoni B type). Palisade formations of nuclei and fibrils were found in approximately one third of the tumors (Fig. 37). In some regions the neoplasms were very cellular, the component cells forming characteristic whorls (Fig. 38).

Neurilemmomas of the autonomic nervous system have been reported by Young and Olafson (236) in 25 brook trout *Salvelinus fontinalis*. The tumors were tiny and not readily visible to the naked eye. The pancreas and mesentery were most extensively involved. The tumors were uniform and characteristic in their structure. A peripheral band of collagen surrounded concentric layers of fibrous tissue; at the center of the growths there often was a group of polyhedral cells with a variable amount of eosinophilic and finely granular cytoplasm. No inclusion bodies were seen, nor were bacteria found in the

TABLE 20: TUMORS OF NERVOUS TISSUES

Species	Site	Author
Codfish *Gadus morhua*	Body wall	Thomas, 1927a
Flatfish *Limanda yokohamae*	Spinal ganglion	Takahashi, 1929
Gilt head *Sparus auratus*	Olfactory plate	Thomas, 1932c
Flatfish *Lepidorhombus megastoma*	Spinal ganglion	Haddow & Blake, 1935
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Snapper *Lutianus griseus*, *Lutianus apodus*, *Lutianus jocu* (76)	Subcutaneous	Lucké, 1942
Brook trout *Salvelinus fontinalis* (25)	Pancreas & mesentery	Young & Olafson, 1944

tissues. The authors believe that the occurrence of the tumors among inbred trout of all ages in a single hatchery suggests that hereditary factors may play a role in the genesis of the neoplasm.

Abstracts of other reported cases of tumors of Nervous Tissues

THOMAS, 1927 a: A tumor, the size of a fetal head, projected from the lateral body wall in the anal region of a codfish *Gadus morhua*. Histologically, the neoplasm consisted of fibrillar tissue among which were scattered islands of ganglion cells. The author believed the fibrillar tissue represented a syncytium of Schwann cells.

TAKAHASHI, 1929: Adjacent to several of the thoracic vertebrae in a plaice *Limanda yokohamae* was a tumor 4 x 2 x 2.5 cm. The growth was soft, gray, and infiltrated the adjacent tissue. It consisted of myelinated nerve fibers among which were many glial and nerve cells. The nerve cells were very large, with relatively small nuclei bearing large nucleoli. The anatomic position and histologic character of the tumor point to an origin in a spinal ganglion.

THOMAS, 1932 c: An adult gilthead *Sparus auratus* bore a very large tumor that reached from the anterior margin of the orbit to the maxilla. Extensive destruction of the nasal and ethmoid bones had taken place; the overlying skin was ulcerated. Epithelial rosettes and acini were present in some regions, lending a pseudocystic appearance to the tissue. Many of the acini were lined by columnar cells that frequently bore tufts of cilia. Among the connective tissue trabeculae were islands of nerve cells that gave origin to neurofibrils, which could be traced throughout the section. The author identified the tumor as a neuroepithelioma of the olfactory plate.

HADDOW and BLAKE, 1933: A tumor that occupied almost the whole thickness of the distal end of the trunk was found in a flatfish *Lepidorhombus megastoma*. Site of origin could not be determined accurately, but it apparently lay in the position of a spinal ganglion. The tumor was composed of a fibrillar groundwork of nervous tissue containing scattered groups of ganglion cells. Osmic acid preparations demonstrated the neurofibrils as nonmedullated. The ganglion cells were imperfect, degenerated, and vacuolated, possessing bipolar continuity with the nerve fibers of the tumor substance.

PICCHI, 1933: A tumor, the size of a pea, occupied the region of a caudal vertebra in a large goldfish *Carassius auratus*. In the periphery of the growth there was a many-layered capsule of pigmented cells; the central region was composed of long fine fibrils that did not stain with van Gieson's reagent; blood vessels were scarce and thin-walled. The author identified the tumor as a schwannoma. The growth did not recur after it was removed. Pieces of the tumor were injected into various cold-blooded animals—fishes, frogs, and salamanders, but in none was there any evidence of growth.

HAMARTOMA

Tumors arising in developmental faults have been recorded only twice in fishes. However, it should be noted that grossly abnormal development is not infrequently observed among fishes raised in hatcheries (124). Similar anomalies have been reported in amphibians (125, 181) and reptiles (34, 75).

Charlton observed a mackerel *Scomber scombrus* in which no pineal organ could be identified and the area was occupied by a series of epithelial folds. In 5 or 6 places, bead-like masses of hyaline cartilage were found among the epithelial cells. The author identified the lesion as a chondro-epithelioma that had destroyed the pineal body but caused little damage to the brain. A tissue malformation, this lesion may perhaps be classified as a hamartoma.

Labbé, in 1930, found a whiting *Merlangus merlangus* in which the normal horizontal convolutions of the intestine were absent, their place being occupied by a tumor. The latter consisted of embryonic epithelial cells separated by connective tissue trabeculae that were poorly vascularized. The author concluded that this was a tumor of embryonic cell rests, *i.e.*, of cells which would have formed the mucosa of the intestine if differentiation had proceeded normally. Like the preceding case, this may represent a local tumor-like developmental fault.

TAXONOMIC DISTRIBUTION OF TUMOR-BEARING FISHES

It is well known that there are distinct differences in natural susceptibility to neoplastic growth for different taxonomic groups. For example, Ratcliffe (168a) in his extensive survey of tumors in captive wild animals and birds found that in mammals the digestive tract, and in birds the genito-urinary organs, are the predominant sites. Moreover, the organ incidence of the various orders, families and species also showed interesting differences. Thus, among birds the grass parrakeet, *Melopsittacus undulatus*, had by far the highest incidence of renal tumors. Many other comparable examples are given for domesticated animals by Feldman (46a).

In fishes, differences in organ or tissue susceptibility are also present. As a class, they are more susceptible to neoplastic processes of their connective tissues than of other tissues. Tumors of nerve sheath are more common in the snapper family, *Lutianidae*, than among other groups; epithelioma of the lip is the most frequent tumor of a species of catfish, *Ameiurus nebulosus*, and multiple osteoma of the haemal spines more often affects the red tai, *Pagrosomus major*, than other species. However, the same kind of tumor may be found in different species of

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fishes, taxonomically and geographically separated. For example, epithelioma of the lip has been reported in both salt and fresh water fishes; among the catfish in the streams of Pennsylvania (122, 136), the barbels in German rivers (97), the tench in a German pond (47), the eels from the brooks and coastal waters of France (217), the stints from the brackish water of the Jasmunder Bay on the Baltic Sea (16), the coalfish in the North Atlantic (228), and the croakers from the South Atlantic (7).

On the contrary, a single species may be affected by a variety of tumors in different organs. For example, in the pike, *Esox lucius,* tumors have been observed in the bones, muscle, subcutaneous tissue, dental structures, ovary, kidney and thyroid; the tumors include osteomas, osteosarcomas, fibrosarcomas, lipomas, adenocarcinomas and lymphosarcomas.

For convenience of reference, all tumor-bearing fishes of which we were able to find records in the literature, or which we have ourselves observed, have been assembled in Table 21. The classification of the fishes is one used by most ichthyologists.* The table is arranged according to orders, families and species of fishes, variety and site of tumor, author and date of publication. Only the zoological names are given; the common names may readily be found by referring to the tables of the various tumors. Inspection of Table 21 shows at once the great preponderance of tumor-bearing bony fishes over cartilagenous fishes; this may be readily accounted for by the numerical superiority and greater economic importance of the former group. Among the bony fishes, nearly one-half of all species affected are representatives of only 5 families, namely the salmoids (*Salmonidae*—salmon, trout), the cyprinoids (*Cyprinidae*—carp, dace, minnows, chubs, barbels), the codfishes (*Gadidae*), the flatfishes (*Bothidae*), and the flounders (*Pleuronectidae*). Members of these families are not likely to be more susceptible to neoplastic growth than other species; rather they are caught in vast numbers for food and hence more often examined.

II. TUMORS OF AMPHIBIANS

Nearly all amphibians belong to one of two orders: the Urodeles which retain their tails throughout life such as the salamanders, and the Anura which are tailed only in the larval stage (frogs, toads). Although frogs and salamanders have been used in laboratories throughout the world for a century, remarkably few tumors have been reported in these animals, perhaps because they have not been looked for by investigators interested in oncology. There is no good reason for believing that amphibians are less susceptible to neoplastic growth than fishes or other vertebrates. For example, systematic search for tumors in one species of the common laboratory frog, *Rana pipiens*, has revealed that these animals are commonly affected with adenocarcinoma of the kidney.

URODELES

In the literature there are only 7 reports dealing with spontaneous tumors in urodeles (Table 21). The recorded tumors comprise carcinomas in 3 species, fibromas in 2, and melanomas in one species. This apparent rarity of tumors in urodeles is the more surprising since these animals, above all other vertebrates, have retained the capacity to regenerate complex tissues such as leg, tail and lens. Various "explanations" have been offered, but none appear biologically sound. It has been suggested, for example, that the ability of the salamander to regenerate lost limbs is evidence of the retention into adult life of such highly potent morphogenetic fields that an aberrant cell will be forced to differentiate by the organizer effect of the adjacent tissues and hence uncontrolled growth cannot occur. The failure of carcinogens to evoke a neoplastic response similar to that observed in mammals has been interpreted by some as evidence that urodeles are more resistant to tumor growth than are other classes of vertebrates. But as stated above the probability is great that tumors are not as rare as they seem to be in these animals; they simply have not been sought, hence have not been found.

Perhaps because of the paucity of spontaneous tumors, few experimental studies of neoplasia have been carried out on these animals. They have been limited largely to attempts at the production of tumors by means of carcinogenic agents, as in the experiments by Martella (132), Koch and his associates (99), Fedotov (46), Finkelstein (51), Shevchenko (186) and Tokin (218). Other experimental studies are those of the Champys (28) on a transmissible epithelioma in the newt, believed to be caused by a virus, and the work of the Brunsts (186a) on melanomas of the axolotl which probably arise on a hereditary basis. A detailed account of these various experiments is given elsewhere (123a).

* We are grateful for advice on this and other matters concerning classification and nomenclature to Dr. Carl L. Hubbs of the Scripps Institution of Oceanography and Dr. Robert R. Miller of the National Museum.

TABLE 21: TAXONOMIC DISTRIBUTION OF TUMOR-BEARING FISHES

ELASMOBRANCHII (Cartilagenous Fishes)			
SELACHII (Sharks; Rays)			
EUSELACHII			
SCYLLIORHINIDAE (Cat Sharks)			
Scylliorhinus catulus	....	Dermal odontoma, skin	Ladreyt, 1929
" canicula	....	Chondroma, skin	Thomas, 1933b
" "	....	Osteoma, skin	Thomas, 1933b
GALEORHINIDAE (Gray Sharks)			
Prionace glaucus	....	Adenoma, liver	Schroeders, 1908
TECTOSPONDYLI			
SQUALIDAE (Dog Fishes)			
Squalus suckleyi	....	Goiter, thyroid	Cameron & Vincent, 1915
" mitsukurii	....	Chondroma, lumbar vertebra	Takahashi, 1929
BATOIDEI			
RAJIDAE (Rays; Skates)			
Raja macrorhyncha	....	Fibrosarcoma, fin ray	Drew, 1912
" clavata	....	Melanoma, skin	Johnstone, 1911
" "	....	Melanoma, skin	Johnstone, 1912
" "	....	Melanoma, skin	Haddow & Blake, 1933
" maculata	....	Hemangioma, skin	Drew, 1912
" batis	....	Melanoma, skin	Johnstone, 1912
" "	....	Melanoma, skin	Johnstone, 1913
PISCES (True Fishes)			
TELEOSTEI (Bony Fishes)			
***ISOSPONDYLI* (Soft-rayed Fishes)**			
ELOPIDAE (Tarpon)			
Elops saurus	....	Chondroma, subcutaneous	Surbeck, 1921
CLUPEIDAE (Herrings)			
Clupea harengus	....	Lymphosarcoma, peritoneum	Johnstone, 1926
" "	....	Rhabdomyoma, trunk muscle	Williams, 1931
" "	....	Leiomyoma, wall of stomach	Thomas, 1933c
Arengus pilchardus	....	Fibroma, peritoneum	Johnstone, 1911
" "	....	Fibroma, peritoneum	Johnstone, 1925
SALMONIDAE (Salmon, Trout)			
Oncorhynchus keta	....	Fibrosarcoma, body wall	Kazama, 1922
" "	....	Fibrosarcoma, body wall	Takahashi, 1929
" "	....	Fibrosarcoma, body wall	Takahashi, 1929
" gorbuscha	....	Fibrosarcoma, body wall	Takahashi, 1929
Salmo salar	....	Goiter, thyroid	Gilruth, 1901
" "	....	Goiter, thyroid	Wilkie, 1902
" "	....	Lymphosarcoma, kidney	Haddow & Blake, 1933
" gairdnerii	....	Goiter, thyroid	Gilruth, 1902
" "	....	Leiomyoma, stomach wall	Plehn, 1906
" "	....	Adenoma, thyroid	Leger, 1925
" "	....	Fibrosarcoma, not given	Leger, 1925
" "	....	Adenoma, liver	Haddow & Blake, 1933
" lacustris	....	Fibroma, subcutaneous	Eberth, 1878
" "	....	Goiter, thyroid	Bonnet, 1883
" "	....	Adenocarcinoma, thyroid	Smith, H. M., 1909
" trutta	....	Adenoma, liver	Plehn, 1909
" "	....	Papilloma, skin	Thomas, 1932e
" "	....	Fibroma, coelom	Kreyberg, 1937
" mykiss	....	Adenoma, intestine	Thomas, 1931a
" carpio	....	Leiomyoma, stomach wall	Pesce, 1907
" clarkii	....	Rhabdomyoma, trunk muscle	Adami, 1908
" several species	....	Adenocarcinoma, thyroid	Gilruth, 1901
" " "	....	Goiter, thyroid	Pick, 1905
" " "	....	Adenocarcinoma, thyroid	Jaboulay, 1908
" " "	....	Goiter, thyroid	Marine & Lenhart, 1910
" " "	....	Goiter, thyroid	Gaylord & Marsh, 1914
" " "	....	Goiter, thyroid	Peyron & Thomas, 1930
" sp.	....	Odontoma, jaws	Plehn, 1912
" sp.	....	Erythrophoroma, skin	Thomas, 1931b

TABLE 21: TAXONOMIC DISTRIBUTION OF TUMOR-BEARING FISHES—Continued

Salvelinus fontinalis	....	Goiter, thyroid	Scott, 1891
" "	....	Fibrosarcoma, trunk muscle	Plehn, 1906
" "	....	Osteoma, fin rays	Thomas, 1932b
" "	....	Neurofibroma, autonomic nerves	Young & Olafson, 1944
Hucho hucho	....	Fibrosarcoma, liver	Plehn, 1909
PLECOGLOSSIDAE			
Plecoglossus altivelis	....	Lipoma, subcutaneous	Takahashi, 1929
OSMERIDAE (Smelts)			
Osmerus eperlanus	....	Epithelioma, lips	Breslauer, 1916
" "	....	Rhabdomyoma, trunk muscle	Bergman, 1921
		APODES (Eels; Morays)	
ANGUILLIDAE (True Eels)			
Anguilla anguilla	....	Adenocarcinoma, left kidney	Schmey, 1911
" "	....	Fibrosarcoma, mesentery	Wolff, 1912
" "	....	Fibrosarcoma, peritoneum	Plehn, 1924
" "	....	Adenocarcinoma, kidney	Plehn, 1924
" "	....	Papilloma, lower lip	Thomas & Oxner, 1930
CONGRIDAE (Conger Eels)			
Conger conger	....	Hemangioma, region of basi-hyal	Drew, 1912
" "	....	Fibrosarcoma, subcutaneous, head	Johnstone, 1920
" "	....	Lymphosarcoma, kidney	Williams, 1931
MURAENIDAE (Morays)			
Muraena helena	....	Adenocarcinoma, palate	Ladreyt, 1935
		HETEROGNATHI	
CHARACINIDAE (Characins)			
Astyanax mexicanus	....	Lymphosarcoma, head	Nigrelli, 1947
		GYMNONOTI	
ELECTROPHORIDAE (Electric eels)			
Electrophorus electricus	....	Papilloma, skin	Coates, Cox, & Smith, 1938
		EVENTOGNATHI	
CATOSTOMIDAE (Sucker)			
Catostomus commersonnii	....	Papilloma, skin	Lucké & Schlumberger, unpubl.
CYPRINIDAE (Carps, Dace, Minnows, Chubs)			
Cyprinus carpio	....	Fibroma, peritoneum	Crisp, 1854
" "	....	Fibroma, mesentery	Gervais, 1876
" "	....	Epithelioma, skin	Dauwe & Pennemann, 1904
" "	....	Epithelioma, not given	Bashford, Murray & Cramer, 1905
" "	....	Osteoma, skull bones	Fiebiger, J., 1909c
" "	....	Epithelioma, fins & operculum	Fiebiger, J., 1909a
" "	....	Fibroma, mesentery	Ronca, 1914
Carassius carassius	....	Fibroma, abdominal wall & mesentery	Plehn, 1906
" "	....	Fibrosarcoma, testicle	Plehn, 1906
" "	....	Chondroma, skull bones	Mulsow, 1915
" "	....	Fibroma, ovary	Freudenthal, 1928
" "	....	Papilloma, skin	Takahashi, 1929
" auratus	....	Fibrosarcoma, dorsal fin	Bland-Sutton, 1885
" "	....	Fibrosarcoma, not given	Semmer, 1888
" "	....	Epithelioma, bladder	Plehn, 1909
" "	....	Fibroma, orbit	Guglianetti, 1910
" "	....	Fibrosarcoma, skin	Schamberg & Lucké, 1922
" "	....	Fibroma, subcutaneous	Wago, 1922
" "	....	Fibrosarcoma, subcutaneous	Johnstone, 1923
" "	....	Lymphosarcoma, kidney	Plehn, 1924
" "	....	Fibrosarcoma, subcutaneous	Roffo, 1924
" "	....	Papilloma, fins	Sagawa, 1925
" "	....	Fibroma, skin	Sagawa, 1925
" "	....	Fibroma, subcutaneous	Eguchi & Oota, 1926
" "	....	Fibrosarcoma, dorsal fin	Dominguez, 1928
" "	....	Fibrosarcoma, skin	Montpellier & Dieuzeide, 1932
" "	....	Neurofibroma, subcutaneous	Picchi, 1933
" "	....	Fibrosarcoma, cutaneous	Lucké, Schlumberger & Breedis, '48

TABLE 21: TAXONOMIC DISTRIBUTION OF TUMOR-BEARING FISHES—Continued

Barbus fluviatialis	....	Epithelioma, lips	Keysselitz, 1908
" "	....	Chondroma, mandible	Surbeck, 1921
" "	....	Adenocarcinoma, thyroid	Schreitmüller, 1924
" vulgaris	....	Epithelioma, lower lip	Clunet, 1910
Gobio several species	....	Papilloma, skin	Schroeders, 1908
" nigronatatus	....	Papilloma, skin	Anitschkov & Pavlovsky, 1923
" blennioides	....	Papilloma, skin	Anitschkov & Pavlovsky, 1923
Idus idus	....	Fibrosarcoma, trunk muscle	Plehn, 1906
Phoxinus laevis	....	Fibrosarcoma, corium near tail	Bugnion, 1875
" "	....	Fibrosarcoma, trunk muscle	Plehn, 1906
" sp.	....	Chondroma, mandible	André, 1927
Tinca tinca	....	Myxoma, orbit	Plehn, 1906
" "	....	Epithelioma, lips	Fiebiger, 1909a
" "	....	Rhabdomyoma, trunk muscle	Kolmer, 1928
Chondrostoma nasus	....	Fibrosarcoma, trunk muscle	Plehn, 1906
Chondrostoma soeta	....	Epithelioma, trunk muscle	Mazzarelli, 1910
Abramis brama	....	Fibroma, coelom	Plehn, 1906
Alburnus lucidus	....	Leiomyoma, trunk muscle	Plehn, 1906
Rasbora daniconius	....	Fibroma, dorsal fin	Smith, Coates, & Strong, 1936
" lateristriata	....	Adenocarcinoma, thyroid	Smith, Coates, & Strong, 1936
" "	....	Lymphosarcoma, peritoneum	Smith, Coates, & Strong, 1936
Danio albolineatus	....	Goiter, thyroid	Klemm, 1927
		NEMATOGNATHI (Catfishes)	
AMEIURIDAE (Horned Pout; Channel Cats)			
Ictalurus catus	....	Epithelioma, lips	McFarland, 1901
Ameirus nebulosus	....	Epithelioma, lips, oral mucosa	Lucké & Schlumberger, 1941
" "	....	Adenoma, kidney	Lucké & Schlumberger, unpubl.
CLARIIDAE			
Clarias dumerlii	....	Adenocarcinoma, thyroid	Schreitmüller, 1924
		HAPLOMI (Pikes; Mud Minnows)	
ESOCIDAE (Pikes)			
Esox lucius	....	Osteosarcoma, anal fin	Walgren, 1873
" "	....	Osteoma, dental plate	Bland-Sutton, 1885
" "	....	Fibrosarcoma, trunk musculature	Ohlmacher, 1898
" "	....	Fibroma, musculature	Plehn, 1906
" "	....	Osteoma, caudal fin	Plehn, 1906
" "	....	Fibrosarcoma, kidney	Plehn, 1909
" "	....	Goiter, thyroid	Marine & Lenhart, 1910
" "	....	Lipoma, subcutaneous	Bergman, 1921
" "	....	Adenocarcinoma, ovary	Haddow & Blake, 1933
" "	....	Lymphosarcoma, subcutaneous	Haddow & Blake, 1933
" "	....	Lymphosarcoma, kidney	Nigrelli, 1947
		CYPRINODONTES (Toothed Carp)	
POECILIIDAE (Top Minnows)			
Platypoecillus maculatus / Xiphophorus hellerii hybrids	....	Melanoma, skin	Haüssler, 1928 & 1934
" "	....	Melanoma, skin	Kosswig, 1929
" "	....	Melanoma, skin	Reed & Gordon, 1931
" "	....	Erythrophoroma, skin	Smith, Coates, & Strong, 1936
" "	....	Melanoma, skin	Gordon, 1937
" "	....	Melanoma, eye	Levine & Gordon, 1946
Platypoecilus maculatus	....	Adenoma, kidney	Jahnel, 1939
Xiphophorus hellerii	....	Fibrosarcoma, retrobulbar	Jahnel, 1939
Heterandria formosa	....	Adenocarcinoma, thyroid	Smith, Coates, & Strong, 1936
CYPRINODONTIDAE (Killifishes)			
Haplochilus chaperi	....	Goiter, thyroid	Klemm, 1927
Jordanella floridae	....	Adenocarcinoma, thyroid	Schreitmüller, 1924
		ANACANTHINI (Codfishes; Hakes)	
GADIDAE (Codfishes)			
Gadus morhua	....	Fibroma, subcutaneous	Bland-Sutton, 1885
" "	....	Fibroma, stomach wall	Bland-Sutton, 1885
" "	....	Osteoma, maxilla	Bland-Sutton, 1885

TABLE 21: TAXONOMIC DISTRIBUTION OF TUMOR-BEARING FISHES—Continued

" "	....	Osteoma, vertebral disc	Bland-Sutton, 1885
" "	....	Hemangioma, pectoral girdle	Murray, 1908
" "	....	Fibroma, oesophagus	Williamson, 1909
" "	....	Fibrosarcoma, subcutaneous	Johnstone, 1912
" "	....	Osteoma, vertebra	Williamson, 1913
" "	....	Fibroma, orbit	Johnstone, 1914
" "	....	Fibrosarcoma, trunk muscle	Johnstone, 1915
" "	....	Fibrosarcoma, subcutaneous	Johnstone, 1923
" "	....	Ganglioneuroma, body wall	Thomas, 1927a
" "	....	Fibrosarcoma, subcutaneous	Thomas, 1927b
" "	....	Fibrosarcoma, ovary	Thomas, 1927b
" "	....	Osteoma, pre-maxilla	Williams, 1929
" "	....	Adenoma, parabranchial body	Peyron & Thomas, 1929
" "	....	Fibrosarcoma, body wall	Williams, 1931
" "	....	Osteosarcoma, pectoral fin	Thomas, 1932b
" sp.	....	Osteosarcoma, operculum	Murray, 1908
" "	....	Fibrosarcoma, mouth	Johnstone, 1920
" "	....	Fibroma, stomach	Johnstone, 1925
" "	....	Hemangioma, swim bladder	Johnstone, 1925
" "	....	Fibroma, duodenum	Thomas, 1933a
Melanogrammus aeglifinus	....	Melanoma, skin	Prince, 1892
" "	....	Fibroma, subcutaneous	Johnstone, 1911
" "	....	Fibrosarcoma, subcutaneous, head	Johnstone, 1922
" "	....	Fibroma, subcutaneous	Johnstone, 1924
" "	....	Adamantinoma, maxilla	Thomas, 1926
Molva molva	....	Adenoma, ovary	Johnstone, 1915
" "	....	Fibrosarcoma, subcutaneous	Johnstone, 1923
" "	....	Hemangioma, body wall	Johnstone, 1925
Pollachius brandti	....	Adenoma, parabranchial body	Takahashi, 1929
" "	....	Adenoma, glandula digitiformis	Takahashi, 1929
" carbonarius	....	Lipoma, liver	Thomas, 1933d
" virens	....	Fibroma, oral cavity	Fiebiger, J., 1909a
" "	....	Rhabdomyoma, mesentery	Fiebiger, J., 1909b
" "	....	Fibrosarcoma, skin	Fiebiger, J., 1912
" "	....	Fibrosarcoma, subcutaneous	Johnstone, 1926
" "	....	Hemangioma, trunk	Johnstone, 1926
" "	....	Fibrosarcoma, mandible	Johnstone, 1926
" "	....	Osteosarcoma, vertebra	Williams, 1929
" "	....	Epithelioma, lower lip	Williams, 1929
" "	....	Chondroma, pre-orbital	Thomas, 1932d
Theragra chalcogramma	....	Adenoma, parabranchial body	Takahashi, 1929
" "	....	Adenocarcinoma, mouth, operculum	Takahashi, 1929
" "	....	Fibroma, body wall	Takahashi, 1929
" "	....	Fibrosarcoma, body wall	Takahashi, 1929
" "	....	Fibrosarcoma, subcutaneous	Takahashi, 1929
" "	....	Osteoma, fin rays	Takahashi, 1929
" "	....	Melanoma, subcutaneous	Takahashi, 1929
MERLUCCIIDAE (Hakes)			
Merluccius merluccius	....	Hemangioma, rectum	Johnstone, 1926
Merlangus merlangus	....	Epithelioma, mandible	Johnstone, 1924
" "	....	Hamartoma, intestine	Labbé, 1930
		HETEROSOMATA (Flounders; Flatfishes)	
BOTHIDAE			
Psetta maeotica	....	Osteoma, vertebral spines	Schroeders, 1908
" "	....	Myxoma, skin, head	Schroeders, 1908
" "	....	Myxoma, skin, trunk & fins	Schroeders, 1908
" "	....	Fibrosarcoma, subcutaneous	Schroeders, 1908
Rhombus maximus	....	Fibrosarcoma, subcutaneous	Johnstone, 1923
" "	....	Fibroma, dorsal fin	Johnstone, 1924
" "	....	Rhabdomyoma, body wall	Young, 1925
" "	....	Fibrosarcoma, body wall	Johnstone, 1927
" "	....	Myxoma, trunk muscles	Williams, 1929
Lepidorhombus megastoma	....	Ganglioneuroma, spinal ganglion	Haddow & Blake, 1933
PLEURONECTIDAE (Flounders)			
Hippoglossus hippoglossus	....	Papilloma, snout	Johnstone, 1912

TABLE 21: TAXONOMIC DISTRIBUTION OF TUMOR-BEARING FISHES—Continued

"	"	....	Fibroma, retroperitoneal	Johnstone, 1913
"	"	....	Fibroma, trunk muscle	Johnstone, 1914
"	"	....	Melanoma, skin	Johnstone, 1915
"	"	....	Fibroma, subcutaneous	Johnstone, 1926
"	"	....	Fibrosarcoma, body wall	Johnstone, 1926
"	"	....	Lipoma, trunk muscle	Williams, 1929
"	"	....	Rhabdomyoma, body wall	Thomas, 1932a
Pleuronectes platessa		....	Myxoma, body wall	MacIntosh, 1908
"	"	....	Fibrosarcoma, subcutaneous	Drew, 1912
"	"	....	Lipoma, body wall	Bergman, 1921
"	"	....	Fibroma, dorsal fin	Johnstone, 1922
"	"	....	Melanoma, pigmented skin	Johnstone, 1925
"	"	....	Papilloma, skin	Johnstone, 1925
"	"	....	Hemangioma, body wall	Johnstone, 1925
"	"	....	Fibroma, subcutaneous, head	Johnstone, 1926
Platichthys flesus		....	Lymphosarcoma, retro-orbital	Johnstone, 1912
Limanda limanda		....	Papilloma, body surface	Johnstone, 1925
Pseudopleuronectes americanus		....	Erythrophoroma, skin	Smith, G. M., 1934
Limandella yokohamae		....	Ganglioneuroma, spinal ganglion	Takahashi, 1929
SAMARIDAE				
Paralichthys olivaceus		....	Lipoma, trunk muscle	Kazama, 1924
"	"	....	Osteoma, haemal spine	Takahashi, 1929
SOLEIDAE (Soles)				
Solea solea		....	Myxoma, peritoneum	Johnstone, 1926
"	"	....	Papilloma, skin	Thomas, 1930

THORACOSTEI (Sticklebacks; Pipe Fishes; Sea Horses)

GASTEROSTEIDAE (Sticklebacks)			
Gasterosteus aculeatus	....	Hemangioma, subcutaneous, head	Plehn, 1906
Spinachia spinachia	....	Epithelioma, skin	Murray, 1908
Pungitius pungitius	....	Hemangioma, iris	Johnstone, 1915

LABYRINTHICI

ANABANTIDAE (Climbing Perch)			
Anabas scandens	....	Papilloma, skin	Fiebiger, 1909a

PERCOMORPHI (Mullets; Barracudas; Tunnyfish; Bass; Snappers; Croakers, etc.)

SCOMBRIDAE (Mackerels)			
Scomber scombrus	....	Hemangioma, body wall	Johnstone, 1924
" "	....	Hamartoma, pineal	Charlton, 1929
Pneumatophorus colias	....	Melanosarcoma, skin	Takahashi, 1929
THUNNIDAE (Tunnies, Albacores)			
Gymnosarda alleterata	....	Erythrophoroma, peritoneum	Thomas, 1931b
TRICHIURIDAE (Hairtails)			
Lepidopus sp.	....	Osteoma, fin rays	Gervais, 1875
MENIDAE			
Menidia beryllina peninsulae	....	Melanoma, subcutaneous	Nigrelli & Gordon, 1944
SERRANIDAE (Sea Basses)			
Serranus scriba	....	Goiter, thyroid	Marsh & Vonwiller, 1916
" cabrilla	....	Goiter, thyroid	Marsh & Vonwiller, 1916
Lateolabrax japonicus	....	Fibrosarcoma, subcutaneous	Takahashi, 1929
" "	....	Fibrosarcoma, pharynx	Takahashi, 1929
Epinephelus gigas	....	Melanoma, branchial region	Dollfus, Timon-David & Mosinger, 1938
LUTIANIDAE (Snappers)			
Lutianus griseus	....	Neurofibroma, subcutaneous	Lucké, 1942
" apodus	....	Neurofibroma, subcutaneous	Lucké, 1942
" jocu	....	Neurofibroma, subcutaneous	Lucké, 1942
SPARIDAE (Porgies)			
Pagrosomus major	....	Osteoma, mandible, haemal spines	Kazama, 1924
" "	....	Osteoma, haemal spines	Sagawa, 1925
" "	....	Adenocarcinoma, body wall	Takahashi, 1929
" "	....	Osteoma, haemal spines	Takahashi, 1929
" "	....	Osteoma, haemal spines	Lucké & Schlumberger, unpubl.
Sparus auratus	....	Neuro-epithelioma, olfactory plate	Thomas, 1932c

TABLE 21: TAXONOMIC DISTRIBUTION OF TUMOR-BEARING FISHES—Continued

GIRELLIDAE		
Box vulgaris	Goiter, thyroid	Johnstone, 1924
SCIAENIDAE (Croakers, Roncadores)		
Pogonias chromis	Epithelioma, lips	Beatti, 1916
" "	Fibrosarcoma, subcutaneous	Beatti, 1916
" "	Osteoma, vertebrae	Beatti, 1916
Micropogon opercularis	Odontoma, upper dental plate	Roffo, 1925
" "	Sarcoma, subcutaneous	Roffo, 1926
SILLAGINIDAE		
Sillago japonica	Fibrosarcoma, base of head	Takahashi, 1929
PLATACIDAE		
Platax pinnatus	Osteoma, vertebral spines	Bell, 1793
" "	Osteoma, vertebral spines	Gervais, 1875
EPHIPPIDAE		
Chaetodipterus faber	Osteoma, first haemal spine	Schlumberger & Lucké, unpubl.
CATAPHRACTI		
SCORPAENIDAE (Scorpion Fishes, Rock Cods)		
Sebastodes inermis	Fibroma, body wall	Takahashi, 1929
Scorpaena porcus	Fibrosarcoma subcutaneous	Schroeders, 1908
HEXAGRAMMIDAE (Greenlings)		
Hexagrammos otakii	Guanophoroma, subcutaneous	Takahashi, 1929
TRIGLIDAE (Gurnards)		
Trigla lineata	Hemangioma operculum	Drew, 1912
Lepidotrigla alata	Fibrosarcoma, body wall	Takahashi, 1929
Chelidonichthys kumu	Allophoroma, subcutaneous	Takahashi, 1929
CHROMIDES		
CICHLIDAE		
Cichlasoma tetracanthus	Epithelioma, skin	Puente-Duany, 1930
Hemichromis bimaculatus	Osteochondroma, operculum	Nigrelli & Gordon, 1946
PHARYNGOGNATHI		
LABRIDAE		
Iridio bivittata	Papilloma, skin	Lucké, 1938
Labrus mixtus	Rhabdomyoma, trunk muscle	Ladreyt, 1930
DISCOCEPHALI		
ECHENEIDIDAE (Remoras, Shark-pilots)		
Echeneis naucrates	Goiter, thyroid	Lucké & Schlumberger, unpubl.
JUGULARES		
CALLIONYMIDAE (Dragonets)		
Callionymus lyra	Lipoma, subcutaneous	Williams, 1929
BLENNIIDAE		
Blennius sp.	Xanthoma, skin	Schroeders, 1908
ZOARCIDAE		
Zoarces viviparus	Melanoma, skin	Bergman, 1921
PLECTOGNATHI		
MONACANTHIDAE		
Alutera schoepfi	Osteoma, interhaemal spine	Schlumberger & Lucké, unpubl.
PEDICULATI		
LOPHIIDAE		
Lophius piscatorius	Melanoma, skin	Ingleby, 1929

Abstracts of reported tumors in Urodeles

VAILLANT and PETTIT, 1902: A Japanese giant salamander *Megalobatrachus maximus* was kept in a Paris zoological garden from 1859 until its death in 1897. The presence of a tumor on the palmar surface of the right forefoot was first noted in 1874. It measured 10 cm. in diameter and had the appearance of a cluster of grapes; the nodules varied from 0.5 to 2 cm. in width. The overlying epidermis was intact and on histologic examination appeared normal. The tumor parenchyma was very vascular and composed of interlacing bundles and whorls of pleomorphic connective tissue cells. The nuclei of the neoplastic cells differed greatly in size, some were large and vesicular, others small with abundant

chromatin. The authors identified the growth as a fibroma.

PICK and POLL, 1903: An elliptical tumor, 14 x 10 x 6 cm., was found to be broadly sessile on the testicle of a Japanese giant salamander, *Megalobatrachus maximus*. The tumor contained many cysts, some of which were as large as an apple. Two firm gray nodules, 1 cm. in diameter, were present near the middle and at the lower pole of the testicle. Histologically the cysts were lined by pleomorphic epithelial cells that formed papillary projections into the cyst cavities. The two smaller masses consisted of small alveoli, clumps, and strands of neoplastic cells that were occasionally found free in vascular spaces; in one area direct invasion of a vein could be demonstrated. The authors identified the tumor as a testicular carcinoma showing vascular invasion and local metastasis.

MURRAY, 1908: An adenocarcinoma arose in the skin glands of a newt, *Triton cristatus*, appearing as a mammillated mass at the angle of the jaw, and as nodules scattered over the tail. The pits seen on the surface of the tumors led into tubules that passed through the epidermal layer of the skin. The neoplasms had their origin in the epithelium of the dermal glands; the cells were pleomorphic, and arranged to form solid sheets, acini, or tubules. The surrounding tissue was not invaded, therefore the author suggested that perhaps the tumors were benign. Nevertheless, throughout his description he refers to them as carcinomas.

KRONTOVSKY, 1916: In an axolotl *Siredon mexicanum*, the author found a "malignant chromatophoroma." It was composed of atypical cutaneous melanophores which were undergoing active proliferation and invading the neighboring tissues. Finkelstein (51), who cited this case, points out that the tumor-bearing animal was found at Kiev in 1916. (See also the comments of Brunst [186a]). During the years 1920–1930 there were in the aquarium of the Kiev Roentgenological Institute axolotls with a hereditary tendency to melanoma. Finkelstein suggests that perhaps these animals are progeny of the original mutant described by Krontovsky.

SCHWARZ, 1923: On the forefoot of a Japanese giant salamander *Megalobatrachus maximus* the author found a pedunculated, almost spherical tumor that measured 2 cm. in diameter. Histologically, it was covered by stratified squamous epithelium, but the bulk of the tumor was composed of dense interlacing bundles of connective tissue; nuclei were not abundant. The neoplasm was rather well vascularized and showed no evidence of malignancy.

CHAMPY and CHAMPY, 1932 and 1935: Studied a carcinoma of the skin in *Triton alpestris*, which was transmissible to other newts of the same species. In 1931 the authors observed that 3 out of 5 newts kept in one tank simultaneously developed cutaneous tumors, which were found to be carcinomas composed of cylindrical and polyhedral cells often arranged as atypical glands. The tumors infiltrated the adjacent tissue. The 3 tumor-bearing animals were removed from the tank (the water of which was not changed) and replaced by 8 other newts belonging to 3 different species. These remained in the "contaminated" tank for one month and were then replaced by another group of 8 animals which remained in the contaminated tank for a month and were then replaced by another group of 8 animals. After 1½ to 2 years, 3 of the 4 in the first group of newts belonging to the homologous species *T. alpestris* developed tumors; individuals of other species were not affected. No tumors developed in the animals placed in the contaminated tank after the first month. Fragments of the tumor were inoculated subcutaneously into several newts; most of the animals died, but, in several, tumors were found about 2 years later at some distance from the site of inoculation. The authors believe that the experiments indicate the existence of a carcinogenic virus.

SHEREMETIEVA-BRUNST and BRUNST, 1948: Among hundreds of axolotls *Siredon mexicanum* the authors in 1932 found a male and a female of the gray variety that had minute black spots on the sides of their bodies. These gradually increased in size and progressed to melanotic tumors. The animals were mated and in successive generations a number of tumor-bearing animals were obtained. The tumors usually appeared first as melanotic areas in the skin at the end of the first year of life. Some of these progressed to tumors, and reached a diameter of several centimeters. The tumors sometimes remained circumscribed, but sometimes invaded the surrounding muscle and connective tissue. The cell of origin appeared to be the melanophore which is normally present in the corium. Histologically the growths resembled the melanomas of hybrid killifishes (q.v.). The tumor was found to be transplantable to other axolotls, either gray or albino. The Brunsts regard this melanoma as arising on a genetic basis.

ANURA

A considerable variety of spontaneous tumors have been recorded in frogs of several genera and

TABLE 22: TUMORS OF TAILED AMPHIBIANS (URODELES)

Species	Tumor	Site	Author
Giant salamander *Megalobatrachus maximus*	Fibroma	Subcutaneous	Vaillant & Pettit, 1902
" " *Megalobatrachus maximus*	Carcinoma	Testicle	Pick & Poll, 1903
Crested newt *Triton cristatus*	Adenocarcinoma	Skin glands	Murray, 1908
Axolotl *Siredon mexicanum*	Melanoma	Subcutaneous	Krontovsky, 1916
Giant salamander *Megalobatrachus maximus*	Fibroma	Subcutaneous	Schwarz, 1923
Alpine newt *Triton alpestris* (3)	Carcinoma	Skin	Champy & Champy, 1935
Axolotl *Siredon mexicanum* (many)	Melanoma	Skin	Sheremetieva-Brunst & Brunst, 1948

TABLE 23: TUMORS OF Tailless Amphibians (ANURA)

Species	Tumor	Site	Author
frog *Rana* sp.	Adenoma	Skin	Eberth, 1868
Meadow frog *Rana virescens*	Osteogenic sarcoma (?)	Femur	Ohlmacher, 1898
European edible frog *Rana esculenta*	Fibroma	Mouth	Vaillant & Pettit, 1902
Leopard frog *Rana pipiens*	Adenocarcinoma	Kidney	Smallwood, 1905
European edible frog *Rana esculenta*	Carcinoma	Ovary	Plehn, 1906
frog *Rana* sp. (2)	Adenocarcinoma	Skin	Murray, 1908
frog *Rana* sp. (2)	Adenoma	Skin	Pavlovsky, 1912
frog *Rana* sp.	Adenocarcinoma	Skin	Pavlovsky, 1912
European edible frog *Rana esculenta*	Hypernephroma	Kidney	Carl, 1913
frog *Rana* sp.	Adenoma	Skin	Pentimalli, 1914
European edible frog *Rana esculenta*	Adenoma	Skin	Secher, 1917/19
European edible frog *Rana esculenta*	Adenocarcinoma	Skin	Masson & Schwartz, 1923
Painted Escuerzo *Ceratophrys ornata*	Fibrosarcoma	Leg	Volterra, 1928
American Bull frog *Rana catesbiana*	Adenocarcinoma	Skin	Duany, 1929
European edible frog *Rana esculenta*	Sarcoma (?)	Leg	Gheorghiu, 1930
Leopard frog *Rana pipiens*	Adenocarcinoma	Kidney	Downs, 1932
Brown-throated frog *Rana fusca* (17)	Adenoma	Skin	Pirlot & Welsch, 1934
Brown-throated frog *Rana fusca*	Myxofibrochondroma (?)	Leg	Pirlot & Welsch, 1934
Leopard frog *Rana pipiens* (1200)	Adenocarcinoma	Kidney	Lucké, 1934, 1938
American Bull frog *Rana catesbiana*	Neurosarcoma	Sacral plexus	Lucké & Schlumberger (not published)
Green frog (tadpole) *Rana clamitans*	Myxosarcoma	Tail	Lucké & Schlumberger (not published)
European edible frog *Rana esculenta*	Hepatoma	Liver	Willis, 1948

species. The reports include adenomas in three species, hepatoma in one, carcinoma in four, sarcoma in three, and fibroma, myxoma and chondroma each in one species.

The more common and up to the present the most extensively studied tumor is the renal adenocarcinoma of the leopard frog, *Rana pipiens* (112, 113, 114). Over 1200 examples of such tumors have been examined in our laboratory since 1933. The tumors occur as solitary or multiple ivory-white masses, and may involve one or both kidneys (Fig. 39). The larger growths not uncommonly metastasize, especially to the liver and lungs (Fig. 40). Histologically, the majority of the tumors have the appearance of adenocarcinomas (Fig. 42), and are thus representative of the most common variety of cancer in man. The component epithelial cells are atypical and usually much larger and basophilic than normal kidney cells (Fig. 41). Mitotic figures are common. The stroma is scanty and poorly vascular. A capsule is lacking and even in very small tumors marginal extensions infiltrate the adjacent kidney. In the larger tumors the entire kidney is often destroyed.

In a relatively small group of the tumors, the appearance is less atypical, and the growths resemble adenomas rather than adenocarcinomas. But there are many gradations, and it is probable that all of these renal tumors are malignant.

A distinctive characteristic of the frog carcinoma is the frequent presence of acidophilic intranuclear inclusions which in their general appearance are like those found in herpes and certain other diseases known to be due to viruses (Fig. 43). In their typical form, they are conspicuous and clearly recognizable. It is obvious, however, that there must be developmental stages and the character of these is still a matter of doubt. The inclusions are invariably confined to the cells of the tumors and do not occur in normal renal epithelium, nor in the cells of other organs. Experiments have supported the indications, first given by the presence of the intranuclear inclusions, that the tumor is in fact very probably caused by a species-specific virus (115).

Our experience with regard to the occurrence of metastasis is worthy of record. In the first series of 158 tumor-bearing frogs no metastases were found, and the conclusion was reached that this cancer, though locally destructive, does not become disseminated. Yet tumor emboli are frequently encountered in vascular spaces (Fig. 44). In a second series of 362 additional cases, metastasis was found in 22 frogs; and to date we have observed over 75 tumor-bearing frogs with metastasis, nearly all of them in animals with relatively large tumors. This experience emphasizes that large series of animals must be studied before an opinion about the character of a neoplasm should be expressed.

The frog tumors have served for many different kinds of experiments, such as manner of growth in the anterior chamber of the eye of homologous and of heterologous species (116, 120). By serial transplantation the tumor has been maintained for 14 generations (176a). It has been transplanted to

tadpoles (19, 22), and studied in tissue culture (117). The frog being poikilothermic lends itself particularly well to studies on the effect of temperature on the tumor as regards its rate and manner of growth (121), and as regards induction and augmentation of metastasis (123b). These experimental investigations are reviewed elsewhere (123a). Here it must suffice briefly to mention a few other studies to indicate the lines of research that have been pursued. Witschi (232) and Briggs (18, 20) have studied the abnormal growths which appear in tadpoles that develop from overripe eggs. Briggs (21) has also investigated the effect on development of eggs exposed to carcinogens, and has introduced carcinogens into tadpoles (17). Duran-Reynals (41) and Schlumberger (176a) have injected several carcinogens into adult frogs.

To the cases reported in the literature two additional anuran tumors may be added. One tumor occurred in a second-year tadpole of the green frog *Rana clamitans*. It formed a mass measuring 7 x 5 x 6 mm. on the dorsal fin, parts of which were destroyed by the growth (Fig. 45). Sections revealed that portions of the dorsal myomeres had been infiltrated and destroyed (Fig. 49). Histologically the tumor had the structure of myxosarcoma, being composed of large stellate and spindle shaped cells which were embedded in an abundant mucinous matrix (Fig. 47). No inflammatory reaction was present. The growth corresponded in every way to a true neoplasm. The tumor is unique in that no neoplasms in larval amphibians have been recorded.

The other tumor occurred in an adult bullfrog in which it formed a swelling in the left flank. On dissection, the growth was found to measure 2 x 1 x 1 cm. It probably had its origin in one of the nerves of the sacral plexus. It protruded into the coelomic cavity, and through the muscles of the back which it had invaded and partly destroyed (Fig. 48). Histologically, the mass was composed of interlacing bundles and whorls which resembled neoplastic connective tissue (Fig. 49, 50). In general appearance the tumor was similar to the more malignant nerve sheath tumors of man.

Abstracts of other reported cases of anuran tumors

EBERTH, 1868: About 60 nodules, small but varying in size, were found on the dorsal and ventral surfaces of a frog. The tumors were located in the corium, were covered by an intact epithelium, and did not infiltrate the adjacent tissues or metastasize. Histologically the tumors were composed of stratified squamous epithelium arranged to form tubules and acini that superficially resembled the normal skin glands. Eberth discussed the possible relation of parasites to the neoplasms, since trematodes and nematodes frequently inhabit the cutaneous glands of frogs. Transplantation experiments yielded negative results.

OHLMACHER, 1898: The left femur of a frog *Rana virescens* presented a spindle shaped swelling that occupied the entire length of the femur and reached a maximum diameter of 8 mm. The periosteum was intact. Histologically the tumor consisted of trabeculae of bone and cartilage surrounded by large spaces. In the latter were rapidly proliferating mononuclear cells that appeared to be derivatives of the bone marrow. The author concluded that the lesion was a "medullary osteosarcoma." However, the description is more suggestive of callus formation after fracture. Murray (141) described a similar lesion in a frog and concluded that it probably represented callus rather than bone tumor.

VAILLANT and PETTIT, 1902: In their report of a tumor in a python the authors briefly mention a fibroma which they found in a frog, *Rana esculenta*. The tumor originated in the buccal cavity and protruded from the mouth.

SMALLWOOD, 1905: Bilateral renal tumors were found in a leopard frog *Rana pipiens* and were interpreted as having arisen in the adrenals and secondarily invaded the kidneys. Histologically the tumor was composed of cuboidal or columnar epithelial cells arranged as dilated and tortuous tubules, into the lumens of which the neoplastic cells often extended as papillary projections. This tumor was first reviewed by Murray (141) who failed to see any resemblance between the tumor cells and those of the normal adrenal. Subsequently one of us (112) obtained a slide of the tumor from Dr. Smallwood. A study of this section revealed that the lesion was a renal adenocarcinoma and bore no relation to the adrenal glands.

PLEHN, 1906: Between the ovaries and fat bodies of *Rana esculenta* were bilateral white cherry-sized tumors; 5 or 6 smaller nodules were scattered among the eggs in the ovaries. The neoplastic cells were large, pleomorphic, with vesicular nuclei; multinucleated giant cells were common. Normal and abnormal mitotic figures were abundant. Plehn believed that the tumors were carcinomas and represented a neoplastic growth of the primitive egg cells.

MURRAY, 1908: A hemispherical nodule was present on the inner aspect of the thigh of an adult male frog. The overlying skin was stretched, but apparently intact. The neoplastic squamous epithelial cells formed irregular tubules and acini separated by delicate connective tissue septae. The epithelium resembled that seen by Eberth in the benign adenomas. In this case, however, the structures were more bizarre and the subjacent muscles were infiltrated by the tumor cells. Murray regarded this growth and another similar one as adenocarcinomas of the skin.

PAVLOVSKY, 1912: The author presented two cases of adenoma and one of adenocarcinoma of the skin in frogs at a meeting of the Russian Pathological Society. The tumors were similar in that the neoplastic tubules communicated with the surface and were lined by tall

columnar epithelium of embryonal type; these cells did not resemble those normally found in the serous or mucous glands of the frog's skin. In the case identified as an adenocarcinoma the tumor cells invaded the surrounding connective tissue.

CARL, 1913: A tumor, about the size of a cherry, projected ventrally from the hilum of the left kidney in an adult female *Rana esculenta.* The growth was round, firm, red-brown, with a slightly uneven surface. The neoplastic cells were pleomorphic, with large nuclei, and resembled those of the adrenal. This appearance was heightened by their occasional arrangement as cords of cells; no glycogen, chromaffin, or fat was demonstrated. Carl identified the lesion as a malignant adrenal tumor; no metastases were found. Attempts to transmit it were unsuccessful.

PENTIMALLI, 1914: Scattered over the skin of the trunk and extremities of a frog were 24 nodular adenomas, the largest of which was the size of a small pea. The overlying skin adhered to some, over others it was ulcerated; the cut surfaces of the tumors were very bloody. Histologically the neoplasms were adenomatous, with frequent papillary proliferations extending into the cysts. No metastases were found; transplants failed to grow.

SECHER, 1917/19: An irregular cauliflower-like mass about 1 cm. in diameter was present on the outer surface of the thigh in *Rana esculenta.* The tumor resembled those described by Murray, though it was less infiltrative and apparently benign. The neoplastic epithelial cells were arranged as solid sheets or acini; the latter occasionally became small cysts. No metastases were present; parasites could not be found.

MASSON and SCHWARTZ, 1923: Three tumors, ranging from 5 to 15 mm. in diameter were located on the right thigh, the right flank, and the lower right abdomen of *Rana esculenta.* They were spherical, pale pink in color, firmly attached to the epiderm but not to the subjacent musculature. Columnar neoplastic epithelial cells lined tubular spaces and were supported by a layer of cuboidal cells. Some of the tubules were cystically dilated; elsewhere the cells formed solid strands. The connective tissue stroma was infiltrated by inflammatory and neoplastic cells which in some instances had penetrated as far as the endothelium of the lymph sacs. The writers were undecided whether the tumors were multicentric in origin or if a single one was the primary growth, the others secondary. Transplants did not grow.

VOLTERRA, 1928: A firosarcoma had produced considerable swelling of the left hind foot in an exotic frog *Ceratophrys ornata.* The tumor was dense, white, and homogeneous in appearance; the overlying skin was ulcerated; the bones of the foot were largely destroyed. Numerous metastatic nodules were present in the liver, two of them were as large as hazel-nuts. The neoplastic cells were spindle-shaped elements gathered into compact bundles; in many places the cells extended to the endothelial lining of the vessels. Mitoses were abundant, and frequently atypical.

DUANY, 1929: Two cutaneous tumors were found in a bullfrog *Rana catesbiana* that had been brought to Havana some years before from the United States. The smaller growth, about the size of the frog's eye, was situated between and slightly behind the eyes. Directly back of this was another tumor as large as the animal's head. The neoplastic epithelial cells formed tubules that somewhat resembled the normal skin glands; the underlying tissues were not invaded. No metastases were found in the viscera, but the author raised the question whether the smaller nodule may have been a metastasis or extension from the larger tumor. He identified the tumor as an adenocarcinoma and noted its similarity to those described by Murray and by Masson and Schwartz.

GHEORGHIU, 1930: The author found a tumor the size of a nut in the right gastrocnemius muscle of *Rana esculenta.* The lesion involved the skin, was soft in consistency, and dull white in color. The cells were pleomorphic, though in some areas those of spindle shape predominated. Filaria as well as numerous bacteria were found throughout the sections. The tumor was easily transplantable and had been carried through 12 generations. About 80 per cent of the grafts "took"; they grew rapidly for from 15 to 20 days, then ulcerated, and the frogs died, probably from infection. The nature of these tumors is questionable; it is quite possible, as Cramer (32) pointed out, that the "tumors" were infectious granulomas.

DOWNS, 1932: A yellow-brown tumor almost filled the coelomic cavity and compressed the liver of *Rana pipiens.* The mass was cystic and surrounded a large part of the intestine. The neoplastic epithelial cells formed bizarre tubules which were identified as of intestinal origin. A section of the tumor was examined by one of us (112) and a striking resemblance to the renal adenocarcinoma noted. That the lesion was actually such a tumor is highly probable since remnants of kidney tissue were seen in the section.

PIRLOT and WELSCH, 1934: The authors found 17 cases of cutaneous adenoma among 1800 frogs *Rana fusca.* The tumors were frequently multiple and varied in diameter from 1 to 15 mm. Stratified columnar epithelial cells with large vesicular nuclei lined the irregular acini. Mitoses were numerous, but the basement membrane was intact and there was no evidence of local invasion or metastasis. Another growth observed by these investigators was a 15 x 7.5 mm. tumor adherent to the overlying skin of the leg in *Rana fusca*; the femur was intact. Masses of spindle-shaped connective tissue cells alternated with regions that were relatively acellular, and composed chiefly of fibrils. Islands of cartilage were present. In some areas a transition from connective tissue to cartilage was apparent. Three frogs were inoculated with the tumor tissue; in all there was a severe inflammatory reaction associated with resorption of the implant. These authors identified this tumor as a myxofibrochondroma, but their description leaves one in doubt as to the nature of the growth.

WILLIS, 1948: A solitary white tumor, 3 cms. in diameter, was found in the liver of an adult European

edible frog *Rana esculenta*. Microscopically, it had an alveolar structure and the general appearance of a well differentiated hepatoma.

Several pseudotumors have been reported in the literature. Gebhart (54) and Wagner (225) described connective tissue hyperplasia of the pylorus of frogs. The first writer implicated coccidia as the etiologic agent, whereas Wagner believed that fibrosis followed encystment of nematode worms and their eggs. Montpellier and Dieuzeide (137) wrote of a localized epithelial proliferation in three specimens of *Discoglossus pictus* that had been kept in the same tank. A characteristic histologic feature was the presence of large eosinophilic cytoplasmic inclusion bodies in the epithelial cells. However, the lesions gradually disappeared, and the authors concluded that they were not true tumors. The disease is somewhat similar to carp-pox, a virus disease of fishes.

III. TUMORS OF REPTILES

In 1908 Murray (141) wrote that no malignant neoplasms had been reported in reptiles up to that time. Scott (183) examined the records of over 6,000 autopsies performed on reptiles of various kinds at the London Zoological Gardens from 1900 to 1925. In only two did he find a neoplasm; both occurred in turtles. Bergman (11) found a macroscopic tumor in only one of 2,200 snakes which he examined; Patay (145a) however, observed a tumor-bearing individual in one of a group of 54 large ring snakes. Our experience with fishes and amphibians leads us to suspect that a careful study of large numbers of reptiles will reveal a tumor incidence not far different from that in other vertebrates.

The reptiles alive today are but a small remnant of the vast numbers that dominated the earth in the Mesozoic age. Among the most spectacular were the giant dinosaurs, in three of which tumor-like lesions were found in the caudal vertebrae. These have been variously interpreted as osteomyelitis, excess callus following fracture, and hemangioma. Moodie (139), who discussed one of these in detail and cited two others reported by Hatcher (71) and Holland (76), believed that they were hemangiomas. However, the descriptions of Hatcher and Holland strongly suggest that the lesions noted by them were bony overgrowths following fracture, rather than neoplasms. Although the dinosaurs were reputedly capable of living for a thousand years, evidence of disease is rarely found in their fossil bones (139). Moodie also reported an osteoma of the third dorsal vertebra in a mosasaur, a large aquatic reptile that lived during the latter part of the Cretaceous.

TURTLES (CHELONIA)

The first tumor in a turtle of which we can find any record is an adenoma of the thyroid reported by Pick and Poll (154) in 1903. It was an egg-shaped, encapsulated mass, 6 x 4 x 2.5 cm., on the ventral aspect of a Brazilian fresh water turtle, *Platemys geoffroyana (Hydraspis hilarii)*. The connective tissue stroma was moderate in amount and divided the parenchyma into elongated lobules. The latter was composed of tubular acini lined by tall columnar epithelial cells which rested upon 2 to 5 layers of small polyhedral cells with relatively large nuclei.

In a brief report on the causes of death in the London Zoological Gardens during 1911, Plimmer (163) records "a glandular cancer in the stomach of a tortoise." A more detailed description of this case was found by Scott (183) among the post-mortem records of the Zoological Society of London. This record identified the animal as an elephantine tortoise in which "there was a large mass, 8 x 5 inches of new growth springing from the mucosa of the stomach which was very red and swollen. The growth was white and gelatinous." There is no account of histologic studies.

In 1913 Plimmer (164) reported the death of a West African fresh water turtle, *Sternothaerus niger*. "Death from rupture of the heart. The heart measured 1¾ x 1¼ inches. The pericardium was full of blood. The heart muscle was converted into a soft whitish growth, full of very large multinucleated cells." Since no mention is made of tumors elsewhere in the body, that found in the heart may

TABLE 24: TUMORS OF TURTLES (CHELONIA)

Species	Tumor	Site	Author
Turtle *Platemys geoffroyana*	Adenoma	Thyroid	Pick & Poll, 1903
Giant tortoise *Testudo elephantina*	Adenocarcinoma	Stomach	Plimmer, 1912
Black terrapin *Sternothaerus niger*	Rhabdomyoma (?)	Heart	Plimmer, 1913
Green turtle *Chelonia mydas* (3)	Papilloma	Skin	Lucké, 1938
Green turtle *Chelonia mydas* (6)	Papilloma	Skin	Smith & Coates, 1938, 1939
Green turtle *Chelonia mydas* (many)	Papilloma	Gall bladder	Smith, Coates & Nigrelli, 1941
Horsfield's tortoise *Testudo horsfeldi*	Fibroadenoma	Lung	Tsvetaeva, 1941
Musk-turtle *Sternothaerus odoratus* (2)	Papilloma	Skin	Schlumberger & Lucké (not published)

have been primary. The presence of the large multinucleated cells suggests that it may have been a rhabdomyoma.

One of us (116) has studied the multiple large papillomas of the skin and eyes in the edible green turtle *Chelonia mydas*. Three specimens, caught off Cape Sable, were available for examination. The tumors were located on the tail, flippers (Fig. 52), the axillae, neck, eyelids, corneas. In one instance bilateral corneal growths had produced blindness (Fig. 51). The tumors were hemispherical and had a rough warty surface which was ulcerated in some areas. They were sessile or had a broad pedunculated base, and ranged in size from small warts a few millimeters in diameter to masses 20 cm. in width. The tumors were rubbery in consistency and on section were white and bloodless. Histologically they were papillomas, with arborescent connective tissue papillae covered by several layers of keratinizing squamous epithelium (Figs. 53, 54).

The connective tissue component became more prominent as the tumor increased in size, and its cellularity gave evidence of active proliferation. Although this never yielded the picture associated with malignant connective tissue tumors, numerous spherical masses, from 3 to 5 cm., were found in the lungs of one of the turtles (Fig. 57). These were composed of dense fibrous tissue covered by ciliated columnar respiratory epithelium (Figs. 58, 59). Whether they were primary tumors of the lung or whether they represented a metastatic dissemination of the neoplastic connective tissue of the skin papillomas cannot be determined with certainty, but an origin *in situ* appears most probable. In this connection may be mentioned the brief note of Tsvetaeva (219) reporting a fibroadenoma of the lung in a Turkestan turtle, *Testudo horsfeldi*. Whether the animal bore any skin papillomas was not stated. Since many papillomas (warts) of man and other animals are of proved virus etiology, it is interesting to consider a similar etiology for these tumors of turtles.

Smith and Coates (191, 192) observed similar fibroepithelial growths in six specimens of *Chelonia mydas*. Besides the more common papillary structures, they also noted occasional smooth elevations which on section were composed of interlacing bands of connective tissue varying in density and covered by a slightly thickened epithelium. The investigators regarded these as fibromas. At times both types of growth coexisted. In a subsequent paper (192) the authors reported that in more than half of 250 tumors occurring in the six turtles examined they found ova of the parasitic trematode *Hapalotrema constrictum* (later [193] reclassified as *Distomum constrictum*). The authors believe that the ova were probably deposited in the preexisting vascular tumor tissue by the migrating flukes, and remained there without affecting the subsequent growth of the tumor. They concluded that the ova were not of primary importance in the etiology of the neoplasm.

Recently, however, Nigrelli (142) reported finding as many as 50 leeches, *Ozobranchus branchiatus*, in various stages of growth on a bit of the papilloma only ½ inch square. The largest leeches measured 10 mm. in length, 5 mm. in width; all were strongly contracted. In the regions where the leeches were attached, the vascularity of the tumors was increased. The hirudin secreted by the leeches may have a direct stimulating effect on the growth of the tumor, or may affect it indirectly by improving the circulation. The author suggested further that the leeches may act as vectors for viruses or other parasites that may be causative in the development of the papillomas.

In these turtles Smith, Coates, and Nigrelli (193) observed a papillomatous disease of the gall bladder associated with the presence in that organ of the trematode *Rhytidodoides similis*. Occasionally the tumors formed discrete patches which surrounded and occluded the orifice of the cystic duct. Not infrequently the entire mucosa was thickened and rugose, the wall edematous and infiltrated with lymphoid elements. These changes were accompanied by capillary dilatation and connective tissue proliferation. The mucosal glands were dilated and tortuous but in none of the 100 affected gall bladders examined was there any evidence of malignant change. Although most of the worms lay free in the bile of the gall bladder, some were found attached to the papillomatous portions of the mucosa. The authors believe that the growths are the result of mechanical or chemical factors associated with the presence of the parasite.

Two musk turtles *Sternothaerus odoratus* bearing skin papillomas were obtained by us from the Philadelphia Zoological Gardens. In one there was a growth, 5 x 5 x 8 mm., on the dorsum of the tail (Fig. 55) and another on the right front flipper. In the second animal a single tumor, 4 x 5 x 5 mm., was present on the left hind flipper. The three small tumors were very firm in consistency and deeply lobulated. On section they closely resembled the common warts of man. A loose, well vascularized connective tissue core was covered by multilayered stratified squamous epithelium showing hyperkeratosis and parakeratosis (Fig. 56). Long pegs of epithelial cells extended deeply into the underlying

connective tissue, but there was no evidence of malignancy. Bits of the tumors were transplanted to the subcutaneous tissue of three musk turtles and three mud turtles, *Kinosternum subrubrum*. After nine months the transplants had failed to grow, and there was no evidence of recurrence at the site of excision.

CROCODILES (CROCODILIA)

Only two instances of neoplasia in the *Crocodilidae* have been reported. In a discussion following the presentation of a paper on tumors in cold-blooded animals by Pick (153) at a meeting of the Berliner medizinische Gesellschaft in 1905, v. Hansemann (72) said, "I wish to add that I saw a lesion, very similar to that just described by Herr Heller in lizards, in a very large crocodile (the species was not given) in our (Berlin) Aquarium. We operated on the animal and it turned out that we were dealing with quite ordinary warts. Soon thereafter the animal died, whether as a result of the operation or from some other cause I cannot say. Nevertheless, I have the impression that the warts per se had nothing to do with its death."

A more detailed account of a neoplasm in a young porose crocodile *Crocodilus porosus* is given by Scott and Beattie (183). The first symptoms were an inability to rise, and a tendency to fall on its right side. A diagnosis of cerebellar tumor was made and the animal was destroyed. At autopsy a tumor somewhat larger than a cherry stone was found on the ventral surface of the cerebellum. On microscopic examination a few glial fibers were seen, but the mass was mainly composed of small round cells and a few larger, often multinucleated cells. Occasional mitotic figures were present; the neoplasm had invaded the adjacent nervous tissue. The entire right auricle of the heart was filled with a tumor that had also invaded the wall of the right ventricle and the interventricular septum. Histologically, it was similar to the growth found in the cerebellum. In the portal spaces of the liver were numerous groups of neoplastic cells; the latter were also diffusely scattered throughout the disorganized parenchyma. The authors concluded that the tumor was a round cell sarcoma, primary in the liver, with metastases in the heart and cerebellum. Examination of the photomicrograph reveals nothing to suggest that the liver cell itself was the neoplastic cell of origin. It may be that the tumor arose in the blood forming tissues (hematopoiesis is active in the liver of some reptiles) and is comparable to lymphosarcoma of man.

LIZARDS (SAURIA)

Because lizards are seldom hunted for food or sport, it is not surprising that the six reports of tumors in these animals deal with individuals kept in captivity. All but one of the lesions were neoplasms of surface epithelium. The exception was a case described by Bland-Sutton (12) of multiple enchondromas in an Indian monitor, *Varanus dracoena*. The animal, which also suffered from rickets, had large tumors in the fifth and seventh cervical vertebrae. Two tumors were present in the distal metaphysis of the right humerus, one in the left humerus. Each of two metacarpal bones bore a tumor near its distal end, and four were found in the hyoid bone. Histologically the neoplasms were composed of hyaline cartilage. It may be noted that the possible etiologic relation of rickets to generalized chondromatosis in man was suggested by Virchow in 1863.

In a male lizard *Lacerta agilis*, Koch (100) found several papillomas on the frontal, parietal, and occipital regions of the head, as well as on the left side of the thorax. The normal shape of the head was altered by the tumors, which were wart-like in character and bluntly conical. They were firm in consistency and on microscopic examination presented the typical appearance of papillomas.

During the discussion of a paper by Pick (153), Heller (74) stated that he had seen keratinized masses in the inguinal regions of several lizards *Lacerta muralis fiumensis*. The lesions attained a considerable height and histologically bore a certain resemblance to the keratinizing condylomata acumenata of man.

Plehn (159) observed similar lesions in another species of lizard, *Lacerta viridis*. The papillomas

TABLE 25: TUMORS OF LIZARDS (SAURIA)

Species	Tumor	Site	Author
Indian monitor *Varanus dracoena*	Enchondroma	Bones	Bland-Sutton, 1885
Lizard *Lacerta agilis*	Papilloma	Skin	Koch, 1904
Lizard *Lacerta muralis fiumensis* (several)	Papilloma	Skin	Heller, 1906
Lizard *Lacerta viridis* (several)	Papilloma	Skin	Plehn, 1910
Tegu *Tupinambis teguixin*	Squamous cell carcinoma	Skin	Schwarz, 1923
Tegu *Tupinambis nigropunctatus*	Squamous cell carcinoma	Mouth	Ratcliffe, Schlumberger, & Lucké (not published)

occurred in several portions of the body and varied greatly in size. Though they occasionally presented firm swellings of considerable dimensions, they never penetrated deeply nor did they infiltrate the surrounding tissues.

A squamous cell carcinoma arising in the skin of the right forefoot of a tegu, *Tupinambis teguixin,* has been reported by Schwarz (180). The tumor was approximately spherical, measured 3 cm. in diameter, and had almost destroyed the metacarpal and proximal two phalanges of the fifth digit. The two proximal phalanges of the fourth toe were also extensively injured. The tumor parenchyma consisted of neoplastic epithelial cells that showed suggestions of keratinization and "pearl' formation. A vascular connective tissue stroma traversed the masses of tumor cells and even appeared to infiltrate the adjacent muscle. Large areas of necrosis were present throughout the neoplasm. Although the overlying skin was intact, the author believes that the tumor arose locally from the epidermal cells. The tumor was operatively removed, but the animal died a few weeks later; no metastases were found at autopsy.

In collaboration with Dr. Herbert L. Ratcliffe, pathologist to the Philadelphia Zoological Garden, we have studied a squamous cell carcinoma of the mouth in a black-spotted teguixin, *Tupinambis nigropunctatus*. When first observed, the tumor, which arose from the left upper gum, had a smooth, lobulated, pink surface and was very firm in consistency. It measured 2.5 x 2 x 1 cm., and during the following months increased fairly rapidly in size, doubling its dimensions in a year. At the end of that time a similar tumor appeared in the corresponding location on the right upper gum. In the following 18 months both tumors increased in size and exhibited central areas of necrosis and ulceration (Fig. 60). At intervals the animal became listless and refused its food. When this occurred a partial excision of the tumors was performed. This was followed by a return of the animal's desire for food.

Several biopsies were taken, the vascularity of the tumor causing considerable bleeding. Attempts at tissue culture of this material were unsuccessful because oral bacteria were a constant contaminant. Transplants were made to the subcutaneous tissue of 23 lizards and the gums of four alligators, but the tumor failed to grow. A similar negative result followed transplantation to the anterior chamber of the eyes of 9 alligators, 9 green frogs, one bull frog, 6 chickens, 12 chicks, and 9 rats.

After being under observation for 2 years and 8 months the animal died from an accidental injury. At autopsy there was local infiltration of the adjacent tissues by the tumor, but no metastases. Histologically the neoplasm was composed of squamous epithelial cells supported by delicate connective tissue trabeculae. The neoplastic cells adjacent to the trabeculae were radially arranged and columnar in shape. Elsewhere they were polygonal, the basophilic cytoplasm was abundant, with prominent intercellular bridges. Frequently cell aggregates were found that resembled the epithelial "pearls" of human squamous cell carcinoma (Fig. 61). The nuclei were pleomorphic, and showed a marked tendency to produce giant forms with one or more prominent nucleoli (Fig. 62). Mitotic figures were numerous.

SNAKES (SERPENTES)

The recorded instances of tumors in snakes are very few, but several are of great interest. One such is the adenocarcinoma of the pancreas of Say's pine snake *Pituophis sayi*, which was reported by Ratcliffe (167). Grossly the pancreas was diffusely enlarged; upon histologic examination the parenchyma was found largely replaced by neoplastic epithelial cells. In a later publication Ratcliffe (168) reported that in a series of 136 snakes which had died in captivity, he found in the pancreas of 45 individuals

TABLE 26: TUMORS OF SNAKES (SERPENTES)

Species	Tumor	Site	Author
Python *Python sebae*	Adenocarcinoma	Ovary	Bland-Sutton, 1885
Python *Python sebae*	Cystic adenoma (?)	Stomach	Vaillant & Pettit, 1902
Ring snake *Tropidonotus natrix*	Adenocarcinoma	Kidney	Patay, 1933
Say's pine snake *Pituophis sayi*	Adenocarcinoma	Pancreas	Ratcliffe, 1935
Water snake *Homalopsis buccata*	Papillary carcinoma	Bile duct	Bergman, 1941
Pine snake *Pituophis melanoleucus*	Melanoma	Skin	Ball, 1946
Pine snake *Pituophis melanoleucus*	Melanoma	Labial folds	Ball, 1946
	Rhabdomyoma	Palate	Ball, 1946
Reticulated python *Python reticulatus*	Melanoma	Skin	Schlumberger & Lucké (not published)
Reticulated python *Python reticulatus*	Melanoma	Skin	Lucké & Breedis (not published)

lesions suggestive of the neoplasm which he had observed in the pine snake. The changes occurred most frequently in the *Crotalidae* (rattle-snakes, water moccasins) and *Colubridae* (pine snakes, black racer). The author traced the development of the lesions to necrotic foci of unknown etiology. The necrosis was apparently followed by an abortive and atypical regeneration of the terminal ductal epithelium, producing small, adenoma-like cell masses. These continued to proliferate while degeneration of acinar and islet epithelium progressed until sometimes the entire organ was replaced by atypical duct epithelium. Whether, or at what stage, the process ceases to be an atypical regeneration and assumes the character of a neoplastic tumor is difficult to determine. At the time of his report Ratcliffe regarded the lesions as early tumors; he has recently informed us that he now considers them to be regenerative rather than neoplastic. Continuing his study of this disease, he examined 261 snakes belonging to the most susceptible species, 60 to 90 days after capture. In contrast to the high incidence of the lesion in animals that had died in captivity only ten of the sacrificed snakes showed evidence of pancreatic neoplasia.

A report by Ball (4) of a malignant melanoma in each of two pine snakes *Pituophis melanoleucus* has recently appeared; we have had the opportunity of examining the tumors. The animals were a male and a female, kept in the same cage at the San Diego Zoological Gardens. In the female snake a dark rapidly growing tumor was noted on the tail after the animal had been in captivity for three years. The tail was amputated, but subsequently two black tumors appeared on the head and one on the abdomen. Two years after the primary tumor was first noted, a fusiform swelling developed anterior to the cloaca. This increased in size and the animal died six months later. At autopsy the tumor measured 11 x 6 x 5 cm. and had encroached on the coelomic cavity. Two metastatic nodules, approximately 2 cm. in diameter, were found in the liver. Histologically the neoplastic cells were spindle shaped with an abundant cytoplasm that frequently contained large numbers of fine pigment granules. The cells were arranged in interlacing bundles; the nuclei occasionally showed some evidence of palisading.

In the male animal a tumor was first noted after almost six years of captivity. The growth, which appeared on the upper labial fold, increased very rapidly in size and the snake was sacrificed 8 months after the tumor was first seen. Histologically it resembled that of the first animal and was identified as a melanosarcoma. No metastases were found. However, a non-pigmented tumor 1.5 cm. in diameter was discovered on the palate. It was composed of large elongated cells with a fibrillar cytoplasm in which cross striations were occasionally seen. Scattered through the section were plump giant cells with multiple, centrally located nuclei. This tumor unquestionably was different from the others; it had all the structural characteristics of a rhabdomyoma.

The melanomas of the pine snakes lend added interest to our recent findings of similar neoplasms in two reticulated pythons, *Python reticulatus*. The first python* was a female, 18 feet in length, and 50 to 60 years of age, which had been on exhibition in the National Zoological Garden of Washington, D. C., for thirteen years, when a nodule the size of a walnut was noticed on the left lateral surface of the trunk. It was located halfway between the head and tip of the tail. During the following year the tumor grew rapidly, and when examined by us measured 11.5 x 8 x 3.5 cm. (Fig. 63). The mass was a variegated red-brown in color; the surface was faintly lobulated. The periphery was covered by expanded scales, but toward the center these were destroyed by a shallow ulceration. The tumor was quite vascular and bled profusely when we removed small portions for examination. It was firm in consistency; on section the surface was a mottled red, yellow, and black. Six months later the tumor had increased somewhat in size and the area of ulceration was larger. The animal was killed as unfit for exhibition.

At autopsy the tumor was found to have penetrated to the vertebral bodies. Its lateral spread had been somewhat restricted by the stout connective tissue septae that normally separate the larger muscle bundles (Fig. 64). Protruding into the coelomic cavity 20 cm. caudal to the heart, was a black, broadly sessile mass that measured 7 x 5 x 6 cm. (Fig. 66) and was covered by smooth transparent mesothelium. On section it was granular, black in color, and the pigment it contained rubbed off and discolored the fixing fluid. On the dorsal surface of each kidney were 6 to 7 widely scattered, roughly circular, umbilicated, yellow-white nodules varying from 0.5 to 1.2 cm. in diameter (Fig. 65).

Histologically the primary neoplasm was composed of interlacing bundles of spindle shaped cells (Fig. 67) which in some areas contained large num-

* We wish to express our appreciation to Dr. W. M. Mann, director of the National Zoological Garden, for placing this animal and the facilities of the Reptile House at our disposal.

bers of pigment granules (Fig. 68). Elsewhere, the cells were less elongated and the nuclei more vesicular, with prominent nucleoli; this appearance was most marked in the metastatic nodules in the kidney (Fig. 69). Occasionally there was a suggestion of palisading (Fig. 70) similar to that observed in the melanomas of the pine snakes. This resemblance of reptilian melanomas to the neurilemmomas of man may therefore perhaps be traced to the origin of both in neurectoderm.

In view of the successful transplantation of mouse tumors to the yolk sac of the chick embryo (201), the finely minced python tumor was inoculated into the yolk sac or onto the chorio-allantoic membrane of 80 embryos. The chick embryos all died within 48 hours after inoculation; in some, death was the result of bacterial infection; in others, the snake tissue appeared to be toxic to the embryo.*

The second python, also a female, was 20 feet long, and had been at the Philadelphia Zoological Garden for 20 years. No information as to age is available, other than that the snake was "full-grown" when received at the Garden. About a year before its death, the keepers noted a swelling in the right side of the upper jaw, and two black tumors on the body. The snake became weak, declined food, and had to be sacrificed.** At autopsy, the tumor was found to arise from the region of the angle of the jaw; it extended forward, was covered externally by unbroken skin, had a firm consistency, and measured 6 x 3 x 2 cm. The cut surfaces of the tumor were pale and had a fibrous appearance. A second tumor, 2 x 2 cm. in diameter, was located on the side of the body, about 8 feet from the tip of the tail. It was somewhat elevated, the covering skin was unbroken; its consistency was relatively soft and its color deep black. A similar, much smaller growth, 0.5 cm. in diameter, was found under the skin about 4 feet from the head. None of the tumors had invaded the neighboring tissues, and no metastases were found. Histologically, the tumors were composed of spindle-shaped cells, grouped in interlacing bundles. Melanin was fairly abundant in the two smaller growths, but the tumor of the jaw was almost amelanotic. The tumors were regarded as multiple non-malignant melanomas. Attempts to transplant them into the anterior chamber of the eye of a number of alligators, turtles and frogs were unsuccessful.

Two other reports of tumors of pythons belonging to a different species are on record. Bland-Sutton (12) observed in a python *Python sebae* which was destroyed at the London Zoological Garden that the viscera were studded with an enormous number of firm yellow-white tumor nodules. The liver contained many similar nodules, varying from the size of a pea to that of a large walnut. In the lung were twenty pea-sized nodules; each kidney bore a mass the size of a walnut, and the ovaries were the seat of several that had attained the size of an orange. Histologically the tumor was identified as a "medullary cancer made up of alveoli containing masses of irregular cells." The source of the metastases could not be determined with accuracy, but the author believed that the ovaries were probably the primary site of the tumor.

More difficult to interpret is the case recorded by Vaillant and Pettit (222) in a python *Python sebae*, which died 8 days after a swelling near the middle of its body first became apparent and about 40 days after the animal had become indifferent to food. At autopsy a spheroidal mass that measured 28 cm. in diameter was found attached to the gastric mucosa and extending into the esophagus. The tumor apparently precipitated intussusception of the small intestine by the pyloric end of the stomach, accounting for the rapid increase in size of the swelling and the death of the snake. The neoplasm was very cystic; though most of the cysts were quite small, one or two contained almost a liter of foul purulent fluid. Connective tissue septae separated the cysts which were often lined by epithelial cells. The nature of this tumor is not recognizable from the authors' description. It may perhaps represent a cystic adenoma, but it may not be a neoplasm. Less difficult to interpret are two older reports of "tumors" in pythons (79, 224). In these cases the growths were almost certainly not true neoplasms; rather, they were probably organized blood clots.

In contrast to the questionable nature of these growths is the carcinoma of the kidney reported by Patay (145a) in a ring-snake, *Tropidonotus natrix*. The animal was a female, 75 cm. in length. The left kidney measured 4 x 2.5 cm. Its lower portion was occupied by a well circumscribed growth, approximately one-third the size of the entire organ. Histologically, the tumor consisted of atypical epithelial cells which in some areas were arranged as acini into which papillary projections protruded; elsewhere the cells were undifferentiated. The author regarded the tumor as a papillary adenocarcinoma, and likened it to similar tumors in mammals.

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* This work was carried out for us through the courtesy of Col. Raymond Randall, director of the Division of Veterinary Medicine, Army Medical Center, Washington, D. C.

** Dr. Herbert Ratcliffe, Pathologist of the Philadelphia Zoological Garden, kindly gave us the snake for examination.

The last of the snake tumors on record is a papillary carcinoma of the bile duct in an East Indian watersnake *Homalopsis buccata*. It was the only tumor found by Bergman in 2200 wild snakes examined (11). He suggests that the apparent rarity of neoplasms in wild snakes may be attributed to the fact that a tumor-bearing individual living under natural conditions would probably be so handicapped that it would fall an easy prey to its enemies. The tumor was a small mass which projected into the lumen of the duct, partly occluding it and infiltrating the wall and the neighboring tissues. Histologically it consisted of atypical pleomorphic epithelial cells. It is of interest that a trematode was found in close proximity to this tumor, because in the gall bladders of other reptiles, green turtles, trematodes have been found associated with papillomatous growths (193).

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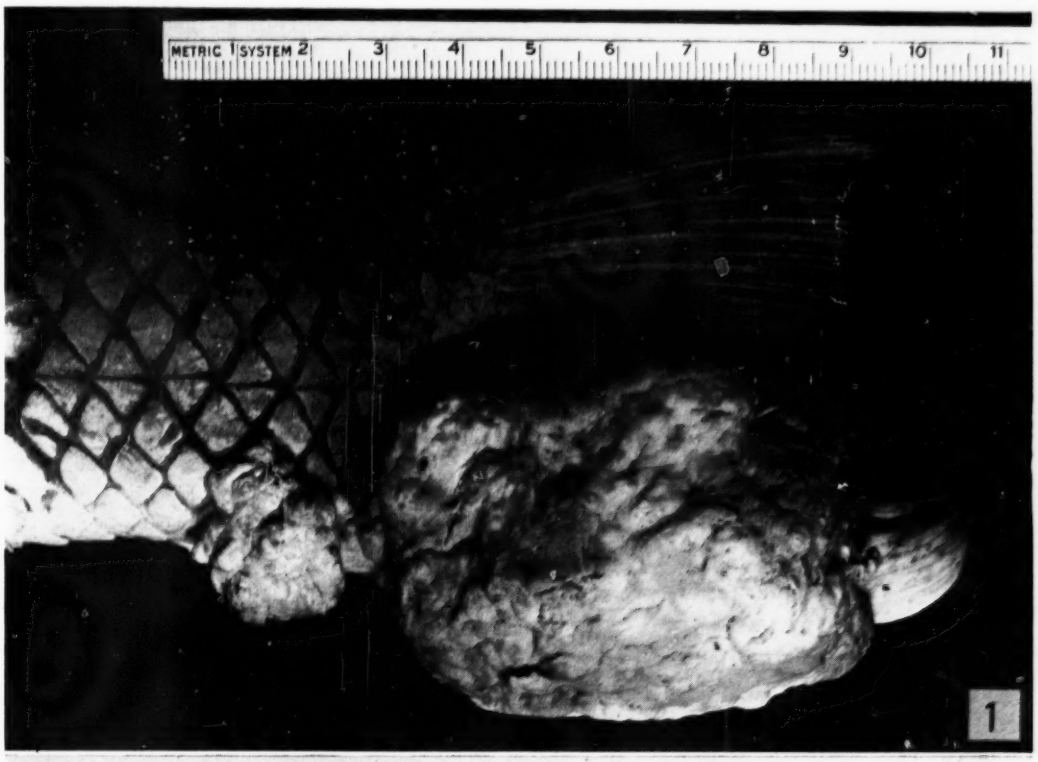
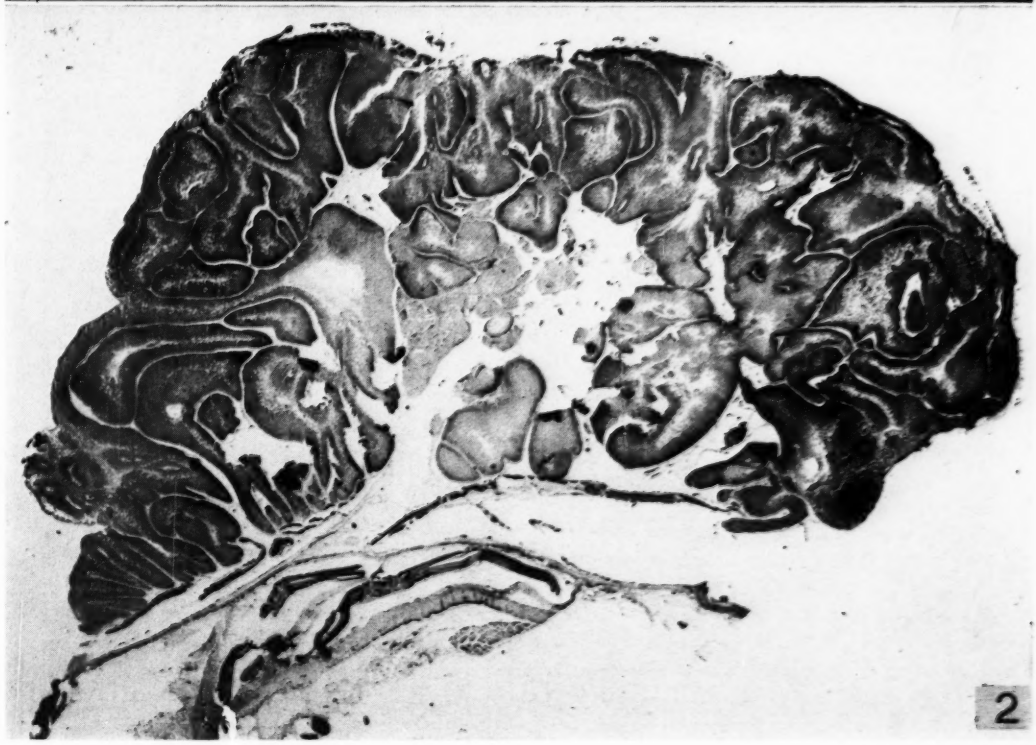
DESCRIPTION OF FIGURES 1 AND 2

Cutaneous papillomas in the common sucker, *Catostomus commersonnii*.

FIG. 1.—A large papilloma occupies the ventral half of the caudal fin; just anterior to it is a smaller tumor.

FIG. 2.—Section through the smaller of the two papillomas. The tumor has a typical papillary arrangement, the supporting connective tissue is scanty. The underlying structures are not involved. For details of microscopic structure see Figures 3 and 4. Mag. × 10.





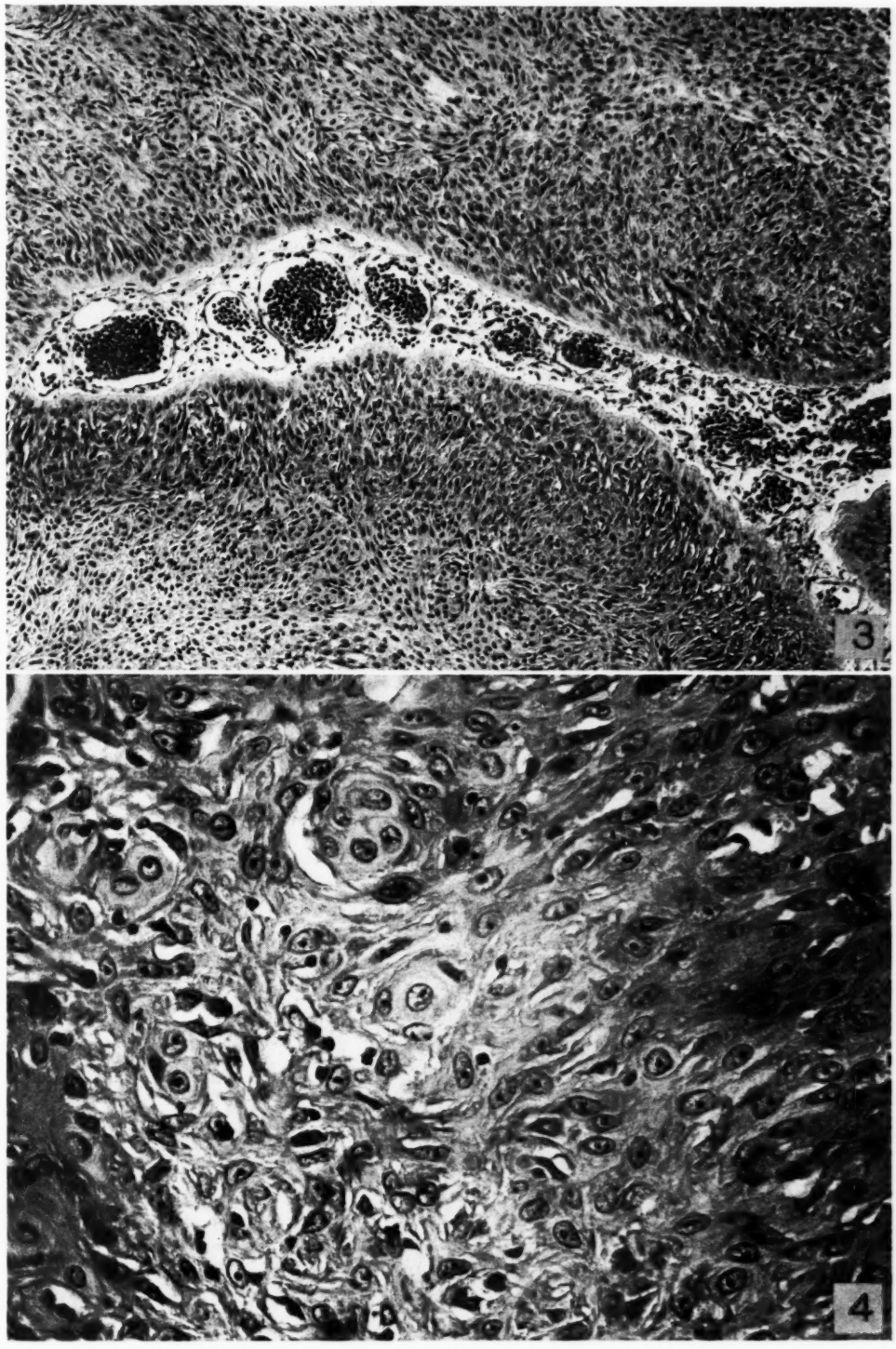
FIGS. 1-2

DESCRIPTION OF FIGURES 3 AND 4

Histologic appearance of papillomas in the common sucker, *Catostomus commersonnii.*

FIG. 3.—Cellular detail from the center of the papillary growth. The epithelial character of the tissue is evident. Some of the cells have acquired a spindle shape, others are arranged in small nests, suggestive of early "pearl" formation. Mag. × 300.

FIG. 4.—A delicate, vascular strand of loose connective tissue is surrounded by solid masses of epithelium. The basal layer of the neoplastic epithelium is composed of columnar cells. Mag. × 80.



FIGS. 3-4

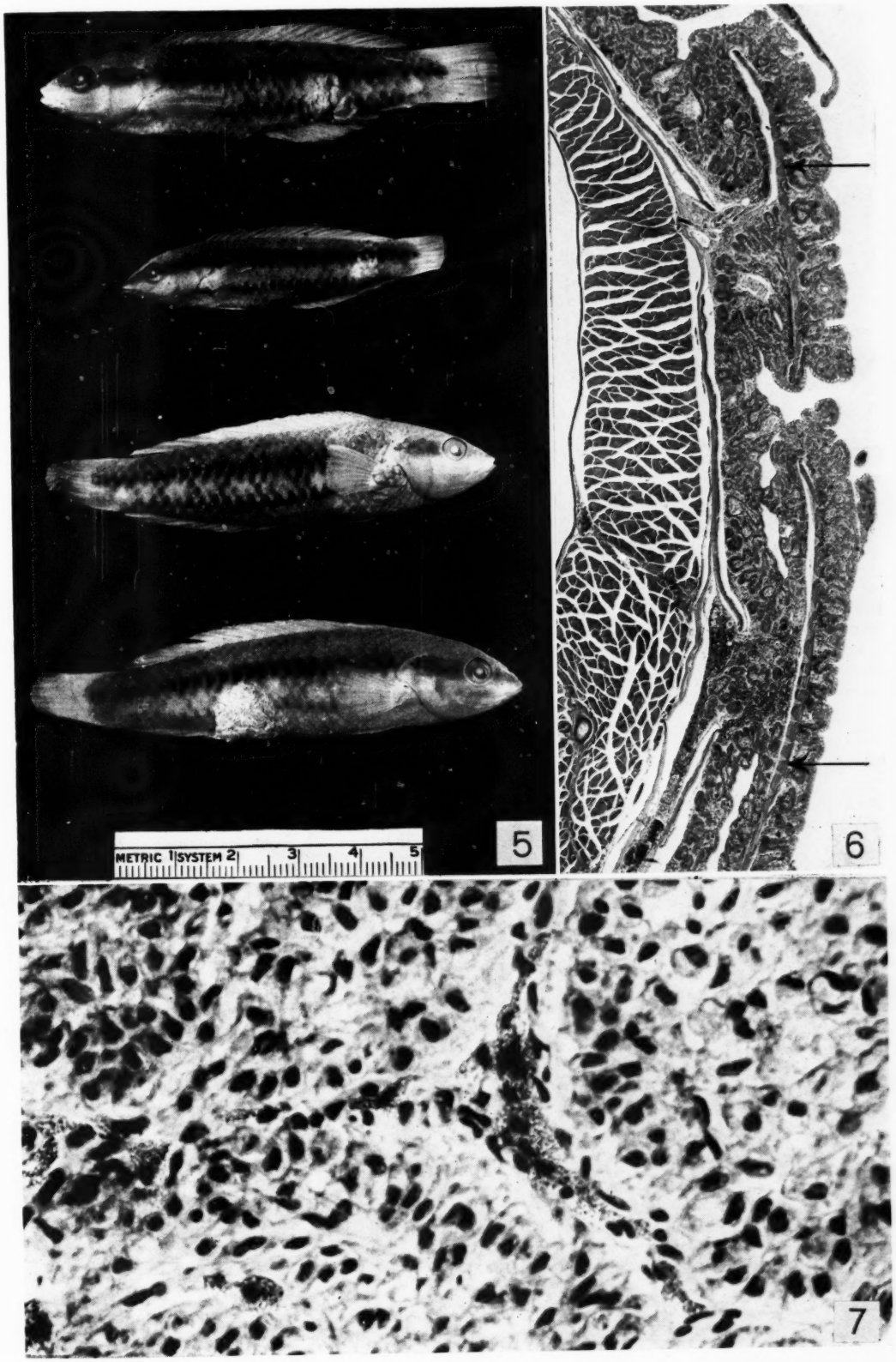
DESCRIPTION OF FIGURES 5 TO 7

Cutaneous papillomas of the slippery dick, *Iridio bivittata*.

FIG. 5.—Gross appearance of the tumors. The two fish at the top of the photograph, and the one at the bottom, bear ventro-lateral tumors at the junction of the middle and caudal thirds of the body. In the fourth fish the papilloma is seen at the base of the pectoral fin.

FIG. 6.—Low power appearance of a papilloma. The tumor is shown in the right half of the figure; on the left is the trunk musculature which is separated from the epithelial tumor by a condensed, but otherwise intact connective tissue. Note the cross sections of two scales (arrows) embedded in the tumor. Mag. × 30.

FIG. 7.—Detail of the tumor showing the finely granular cytoplasm and small nuclei of the epithelial cells. The supporting connective tissues is scant in amount. Mag. × 500.



FIGS. 5-7

DESCRIPTION OF FIGURES 8 TO 11

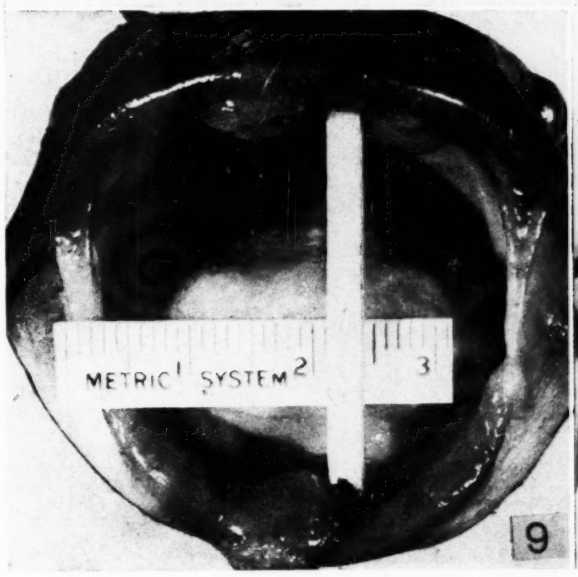
Epithelioma of the lip in catfish, *Ameiurus nebulosus*.

FIG. 8.—Epithelioma of the lower lip with extension to the upper lip, dental plates, and floor of the mouth.

FIG. 9.—Apposition tumors near the midline of the upper and lower lip and dental plates. To the right of the midline on the upper dental plate is a third tumor.

FIG. 10.—A large epithelioma occupies the entire lower lip, dental plate, anterior portion of the floor of the mouth. Externally it forms a large pendulous mass that interferes with feeding.

FIG. 11.—Apposition tumors of the upper and lower lips.



FIGS. 8-11

DESCRIPTION OF FIGURES 12 TO 15

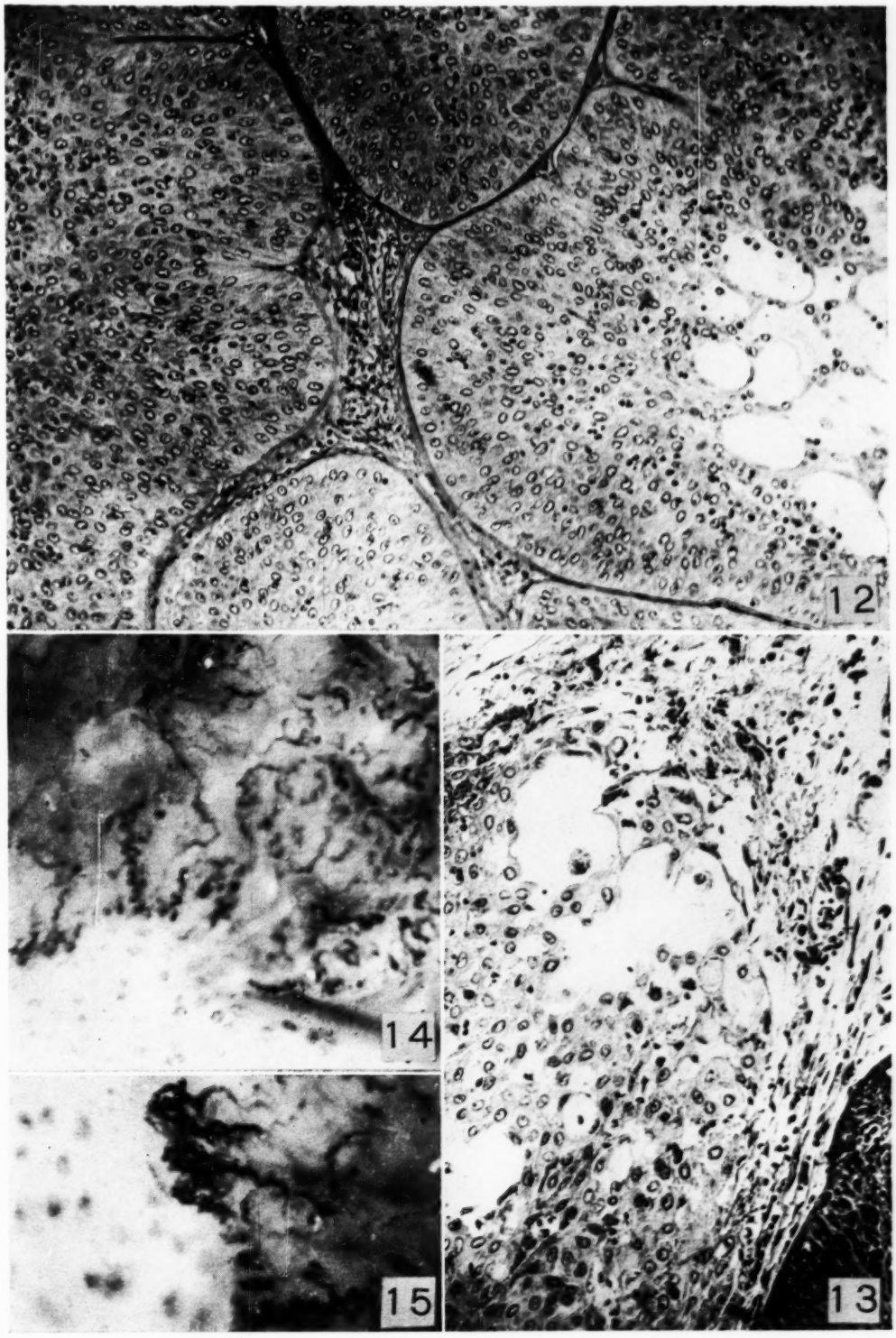
Histologic appearance of epithelioma of catfish, *Ameiurus nebulosus*

FIG. 12.—Sections through a representative tumor showing compact masses of epithelial cells which are supported by a scanty vascular stroma. Mag. × 170.

FIG. 13.—Invasion of the adjacent connective tissue by neoplastic epithelial cells. Mag. × 200.

FIG. 14.—Vascular bed of the living tumor (the upper two-thirds of the photograph). Note the dilatation and tortuosity of the vessels in the tumor as compared with the delicate capillary loops in the normal tissue (lower third of photograph). Mag. × 10.

FIG. 15.—Vascular bed of the living tumor at the margin of a growing epithelioma. The distended vessels in the tumor are seen in the right half of the figure. Note the abrupt transition between the vessels in the neoplastic area and the capillary loops of the normal lip on the left. Mag. × 30.



FIGS. 12-15

DESCRIPTION OF FIGURES 16 TO 19

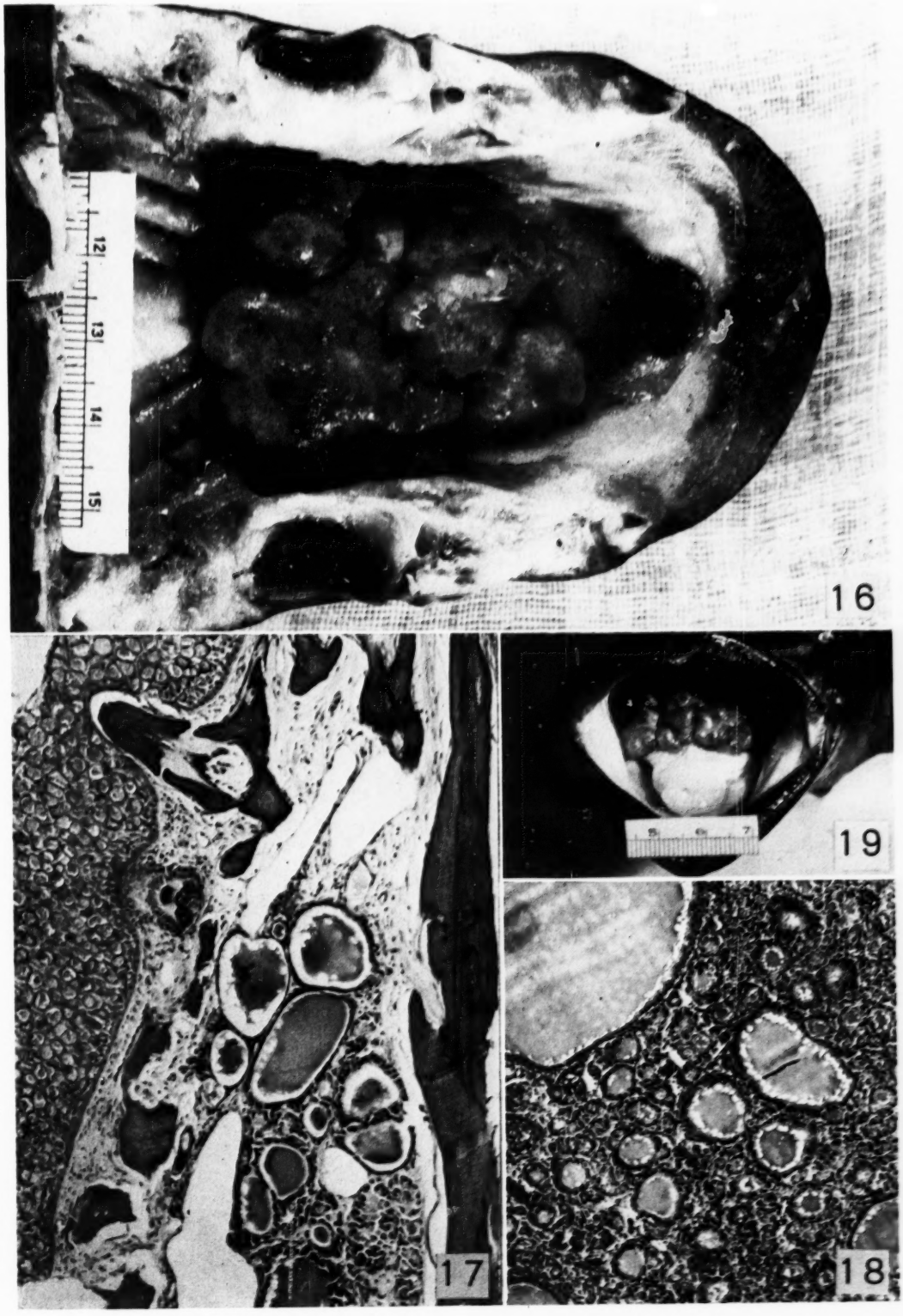
Thyroid "tumors" in the shark sucker, *Echeneis naucrates*.

FIG. 16.—Gross appearance of a tumor protruding through the floor of the mouth. The top of the head has been removed by a section which passed through the level of the eyes (visible in the photograph as bilateral heavy black crescents).

FIG. 17.—Thyroid tissue between the bony lamellae of a branchial arch. On the left is normal epidermis. This apparently aberrant location of thyroid tissue is normal for teleost fishes. Mag. × 100.

FIG. 18.—Microscopic appearance of tumor shown in Fig. 16. Many of the acini are very small, with only a tiny lumen lined by tall columnar cells. As the acini increase in size the lining epithelial cells become flattened, and the amount of colloid increases. There are many marginal vacuoles in the colloid. Mag. × 70.

FIG. 19.—Gross appearance of a tumor on the floor of the mouth in the living fish.



FIGS. 16-19

DESCRIPTION OF FIGURES 20 TO 24

Subcutaneous "fibrosarcoma" in goldfish, *Carassius auratus*.

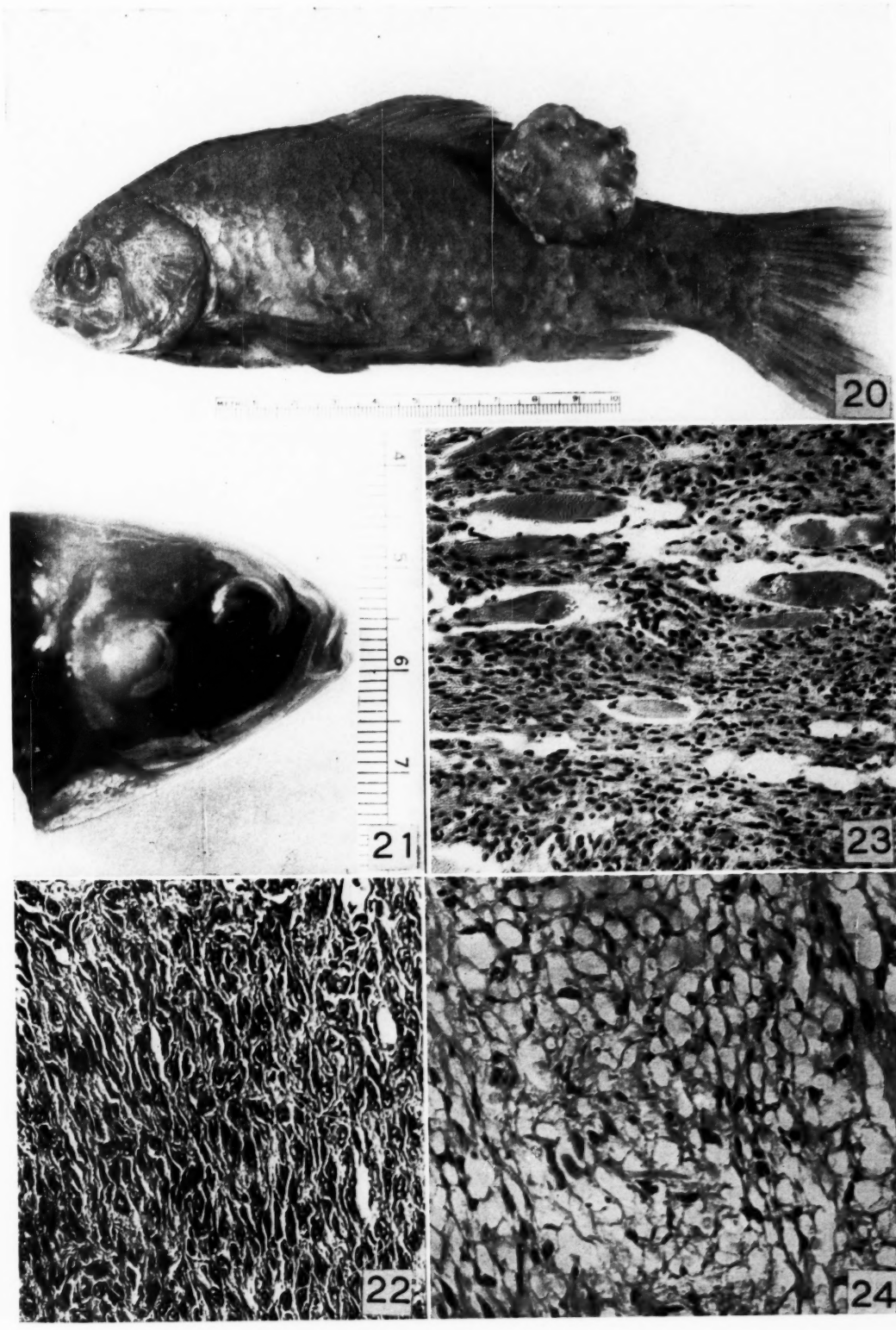
FIG. 20.—Gross appearance of a large tumor located on the side of the body.

FIG. 21.—Gross appearance of a tumor present on the right operculum near its posterior margin.

FIG. 22.—Histologic pattern of the tumor showing spindle shaped neoplastic cells in compact arrangement. Mag. × 300.

FIG. 23.—Infiltration of the trunk musculature by tumor tissue. Mag. × 200.

FIG. 24.—An area in which the neoplastic cells have become stellate in shape, resembling those of a myxoma. Mag. × 380.



FIGS. 20-24

DESCRIPTION OF FIGURES 25 TO 28

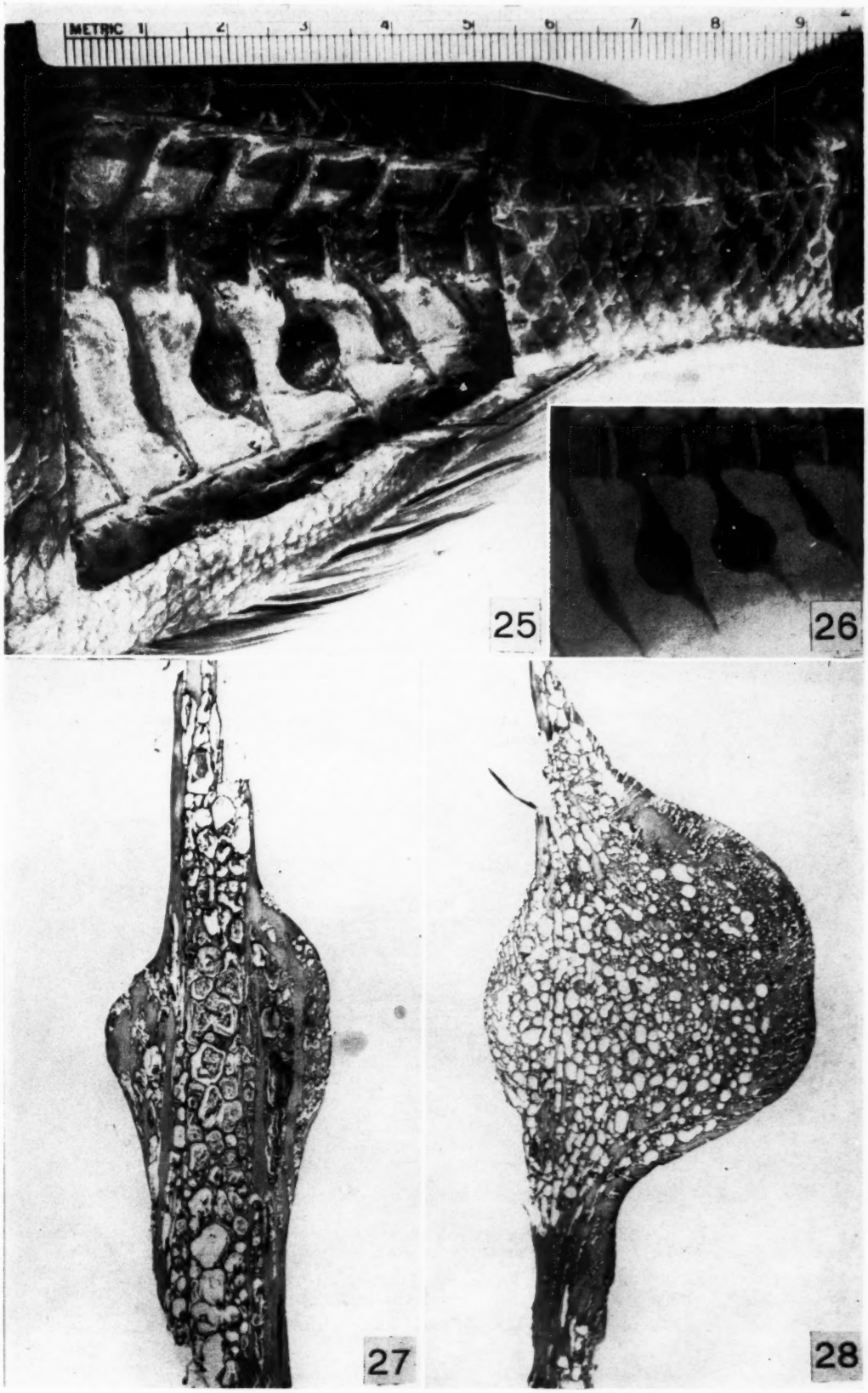
Multiple osteomas in the red tai, *Pagrosomus major*.

FIG. 25.—Dissection showing the ventral (haemal) spines arising from the caudal vertebrae. The 2nd, 3rd, and 4th spines in the figure bear osteomas.

FIG. 26.—Roentgenogram of the tumors shown in the previous figure, made before dissection had been carried out.

FIG. 27.—Longitudinal section of the smallest of the tumors shown in Figure 26. New bone formation is entirely subperiosteal. Mag. × 8.

FIG. 28.—Longitudinal section of the largest of the three osteomas shown in Figure 26. There is a marked central expansion of the spine by new formation of atypical bone. Mag. × 6.



FIGS. 25-28

DESCRIPTION OF FIGURES 29 TO 33

"Osteomas" of the angelfish, *Chaetodipterus faber,* and on the file fish, *Alutera schoepfi.*

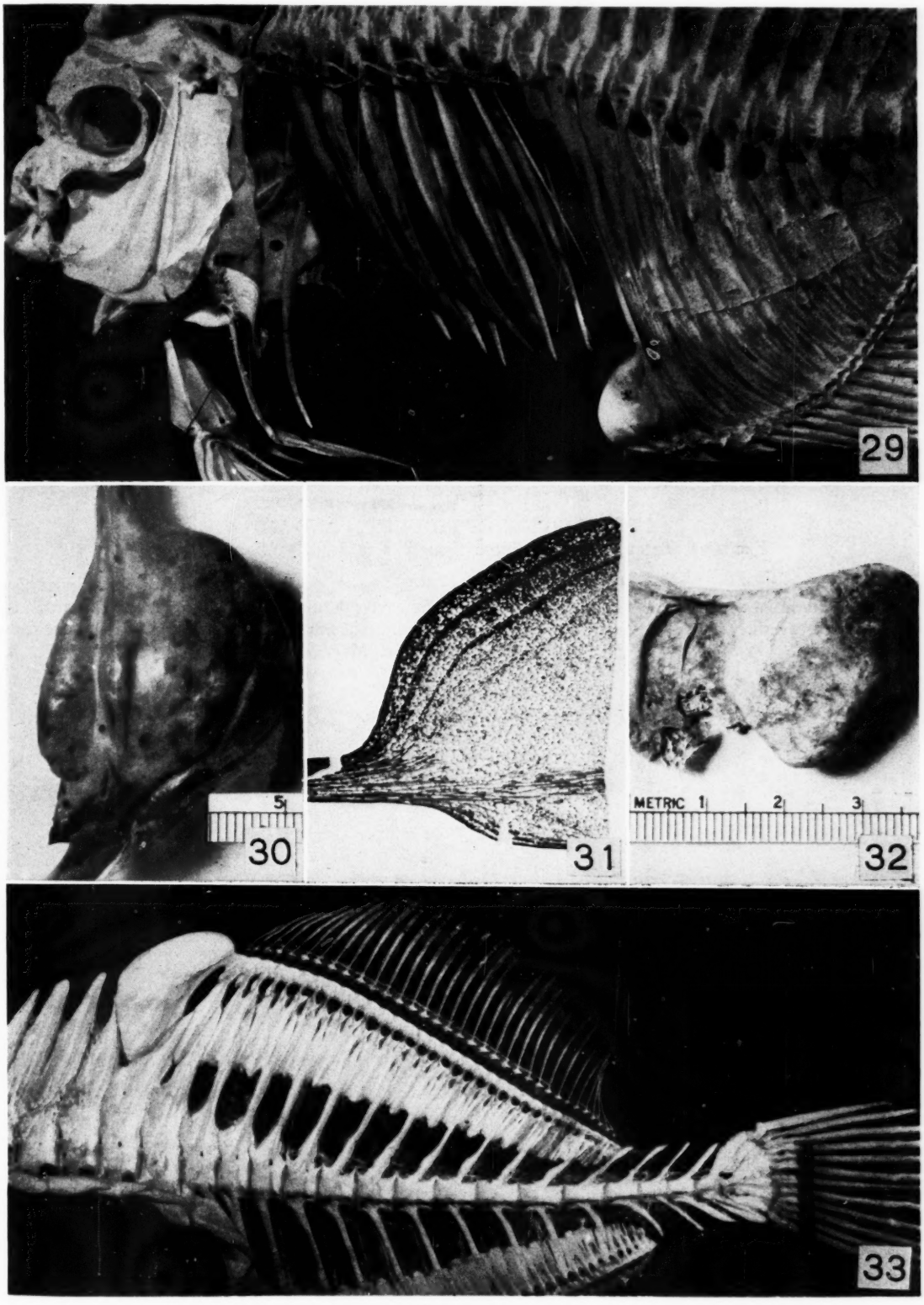
FIG. 29.—Osteoma of the first ventral (haemal) spine of an angelfish. The tumor is marked by an asterisk. One-half natural size.

FIG. 30.—Another example of osteoma of the first haemal spine of an angelfish.

FIG. 31.—Longitudinal section through the osteoma shown in the previous figure. Note the "annular" rings indicating periods of arrested growth, probably seasonal. Mag. × 2.

FIG. 32.—Osteoma of a bone (cleithrum) of the shoulder girdle in an angelfish. The specimen was found in a Florida Indian kitchen-midden.

FIG. 33.—Osteoma of the first interneural spine (just anterior to the dorsal fin) of a file fish. One-half natural size.



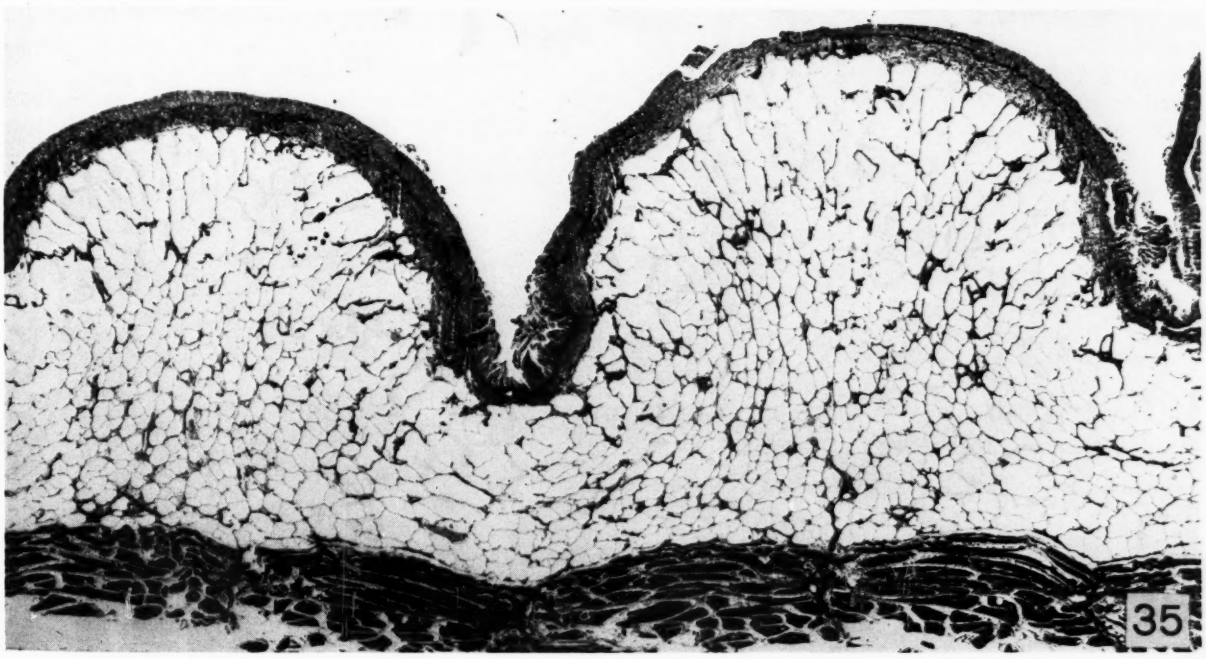
FIGS. 29-33

DESCRIPTION OF FIGURES 34 AND 35

Excessive accumulation of adipose tissue in a catfish, *Pygidium totae*.

FIG. 34.—Gross appearance of symmetrical, deeply lobulated, subcutaneous massive accumulations of adipose tissue in a South American catfish.

FIG. 35.—A section through the skin, adipose tissue, and trunk musculature. The overlying skin is normal, as is also the subjacent muscle. The adipose tissue is composed of adult fat cells. Mag. × 10.



FIGS. 34-35

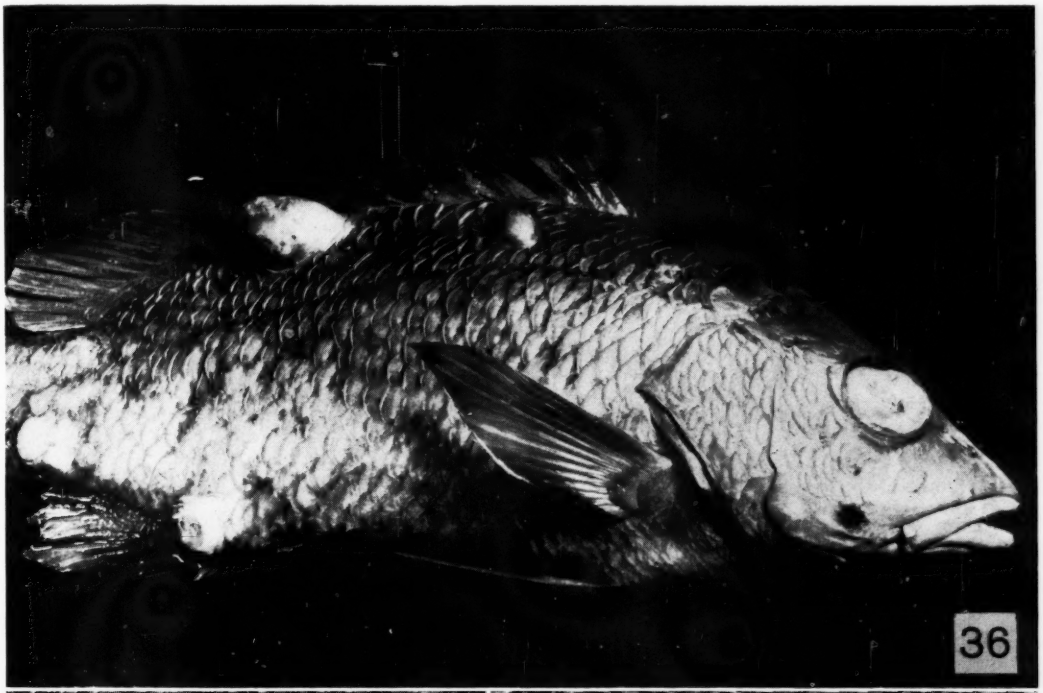
DESCRIPTION OF FIGURES 36 TO 38

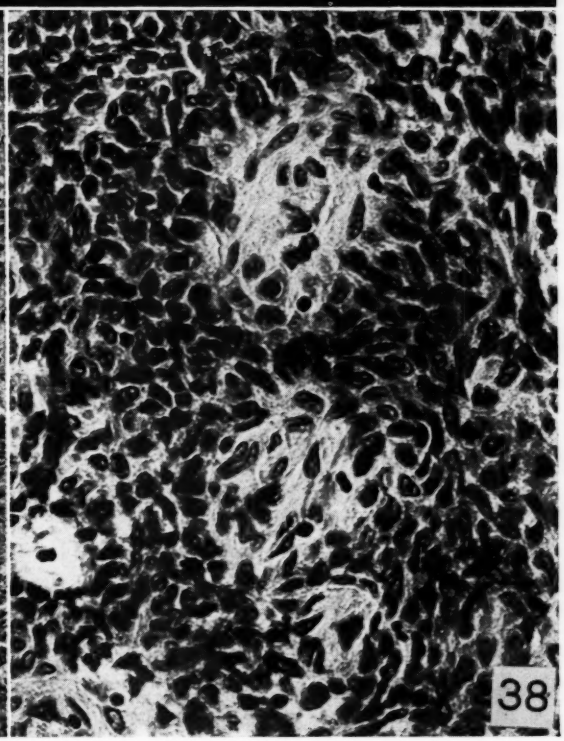
Tumors of the nerve sheaths in fish of the snapper family, *Lutianidae*.

FIG. 36.—Gross appearance of multiple tumors. Two tumors are present on the back, between the dorsal fin and the lateral line, a third occupies the base of the right pelvic fin, and the fourth is on the ventro-lateral aspect of the tail. One-half natural size.

FIG. 37.—Relatively acellular areas composed of fibrils alternate with crowded collections of nuclei, thus giving a "palisading" appearance. Mag. × 280.

FIG. 38.—Highly cellular region in which the nuclei are arranged in whorls about central bundles of fibrils. Mag. × 560.





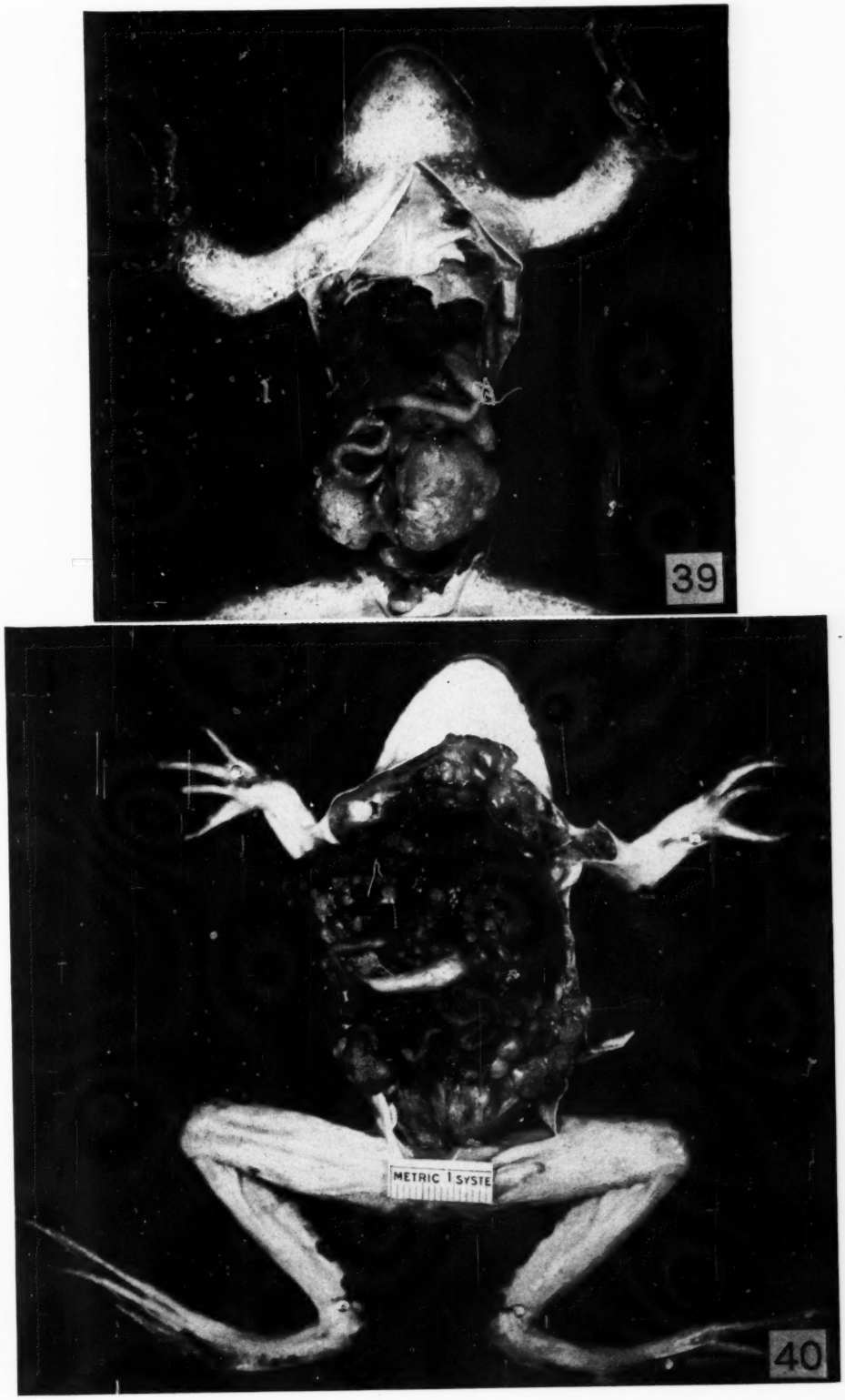
FIGS. 36-38

DESCRIPTION OF FIGURES 39 AND 40

Adenocarcinoma of the kidney in the leopard frog, *Rana pipiens*.

FIG. 39.—Bilateral renal adenocarcinomas that have almost completely replaced both kidneys.

FIG. 40.—Multiple metastases to the liver, pancreas, and ovaries from bilateral kidney tumors.



FIGS. 39-40

DESCRIPTION OF FIGURES 41 TO 44

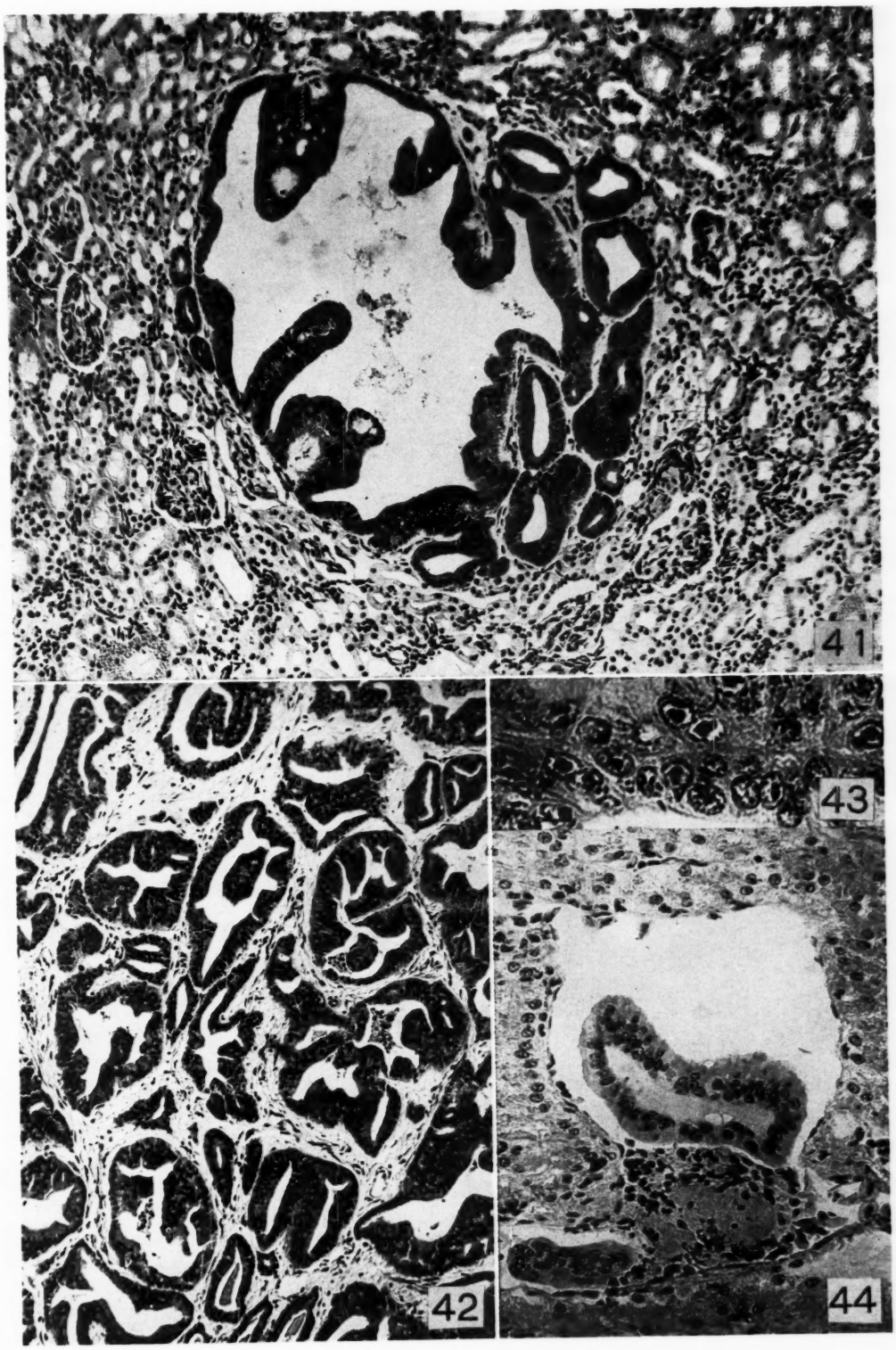
Histologic appearance of renal adenocarcinoma of the leopard frog, *Rana pipiens*.

FIG. 41.—Very early, solitary, cystic tumor surrounded by normal kidney tissue. Compare the size of the neoplastic tubules with the much smaller ones in the adjacent normal kidney. Mag. × 95.

FIG. 42.—Microscopic appearance typical of the majority of the tumors. The neoplastic epithelium forms bizarre tubular or acinar structures supported by a moderately vascular connective tissue stroma. Mag. × 70.

FIG. 43.—Intranuclear inclusions in neoplastic cells. The chromatin is condensed about the periphery of the nucleus, and is separated from the large central acidophilic inclusion by a clear space. Mag. × 400.

FIG. 44.—Two large tumor emboli in vascular spaces within the liver. Mag. × 200.



FIGS. 41-44

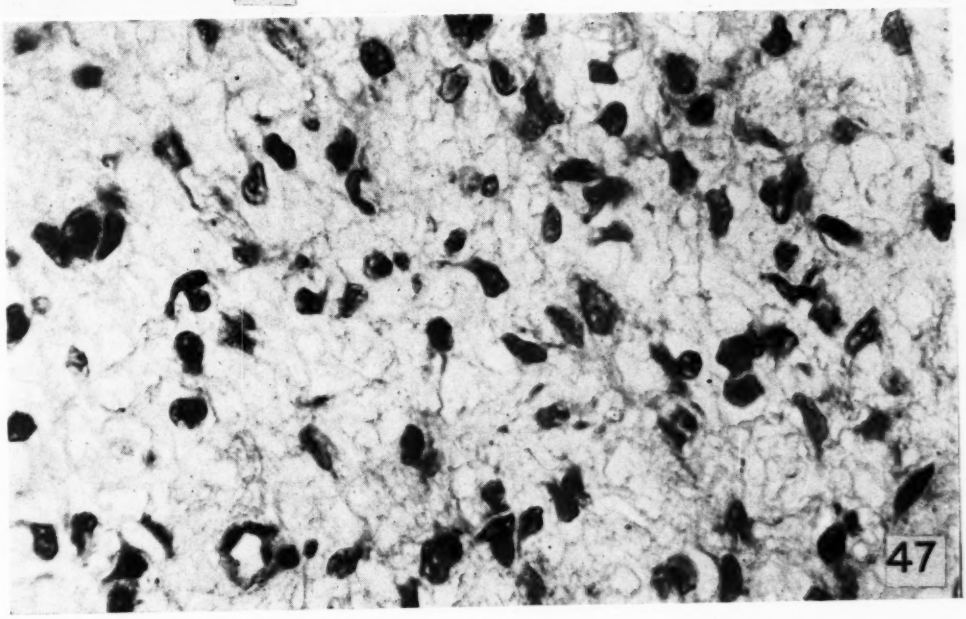
DESCRIPTION OF FIGURES 45 TO 47

Tumor in a tadpole of the green frog, *Rana clamitans*.

FIG. 45.—A coarsely lobulated tumor on the dorsum of the tail, near its base.

FIG. 46.—Section through the neoplastic region. The neoplasm apparently arose in the subcutaneous mesenchyme and is infiltrating the dorsal myomeres. Mag. × 7.

FIG. 47.—The bulk of the tumor is composed of loosely arranged stellate cells such as are characteristic of a myxoma or myxosarcoma. Mag. × 400.



FIGS. 45-47

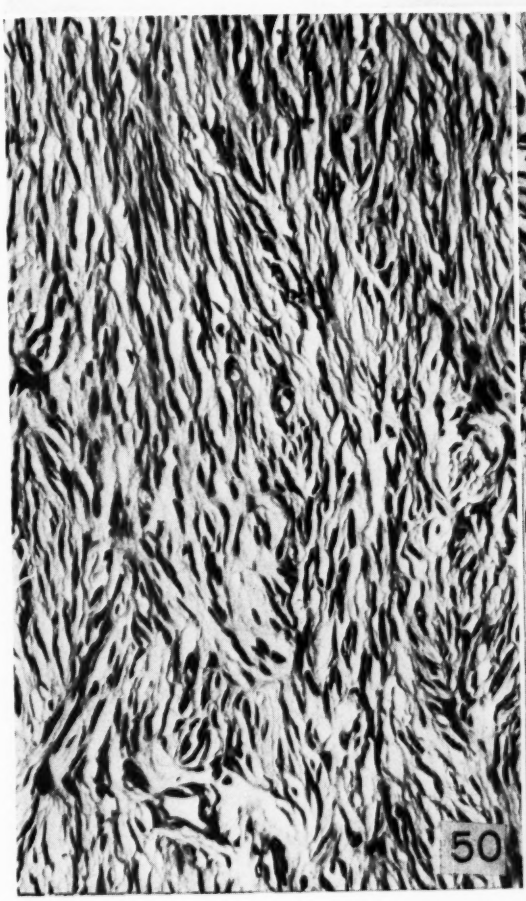
DESCRIPTION OF FIGURES 48 TO 50

Sarcoma in a bullfrog, *Rana catesbiana*.

FIG. 48.—Gross appearance of the legs and posterior half of the trunk of a bullfrog. At the junction of the left thigh and trunk is a roughly spherical tumor, half of which lies within the coelomic cavity (white arrow); the remainder has penetrated the back muscles and forms a prominent subcutaneous mass (black arrow).

FIG. 49.—Interlacing bundles and whorls of delicate connective tissue make up the bulk of the tumor. Mag. × 150.

FIG. 50.—At the periphery of the neoplasm the fibrils are arranged in parallel bundles; palisading of the nuclei is not present. Mag. × 150.



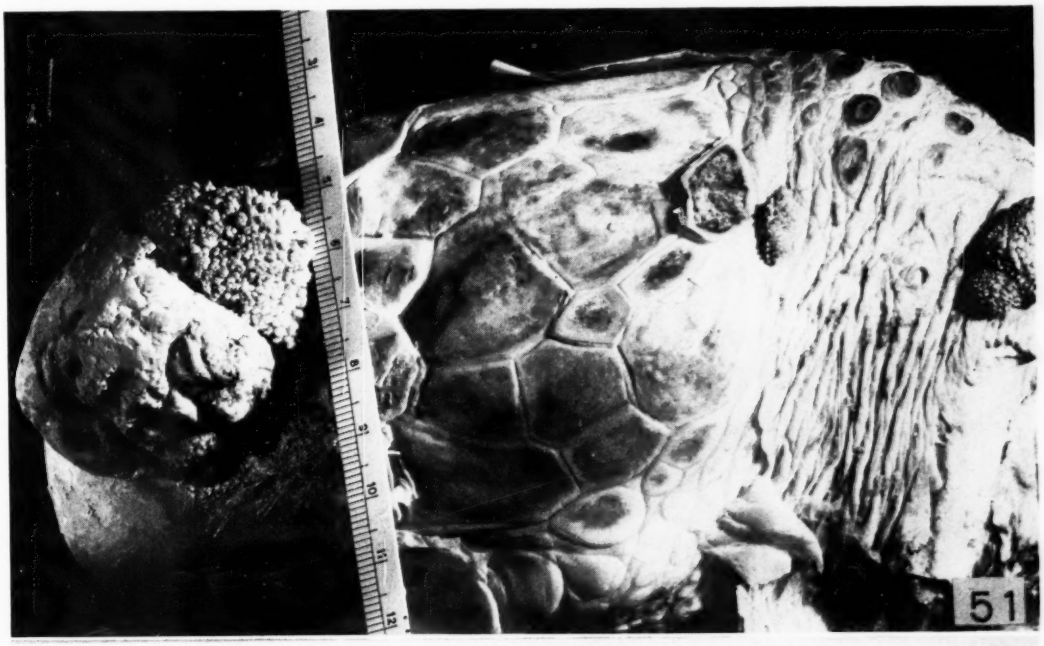
FIGS. 48-50

DESCRIPTION OF FIGURES 51 AND 52

Cutaneous papillomas of the green turtle, *Chelonia mydas*.

FIG. 51.—Head and neck of turtle. At the left of the photograph a large lobulated tumor completely covers the eye. Two small papillomas are present on the dorso-lateral aspect of the neck. (Natural size).

FIG. 52.—Flipper of turtle. In the axilla is a large spherical tumor, portions of which are hemorrhagic and necrotic. (One-half natural size).



FIGS. 51-52

DESCRIPTION OF FIGURES 53 TO 56

Histologic appearance of the papillomas of the green turtle, *Chelonia mydas*.

FIG. 53.—The hyperplastic epithelium of a cutaneous papilloma rests on a dense supporting stroma. Mag. × 150.

FIG. 54.—Arborescent papilloma growing on the cornea. Mag. × 5.

Papilloma of the musk turtle, *Sternothaerus odoratus*.

FIG. 55.—Coarsely lobulated papilloma on the dorsum of the tail. Mag. × 4.

FIG. 56.—Section of the papilloma showing hyperkeratosis and parakeratosis of the epithelium. Long pegs of epithelial cells penetrate deeply into the supporting stroma of loose, well vascularized connective tissue. Mag. × 70.

FIGS. 53-56

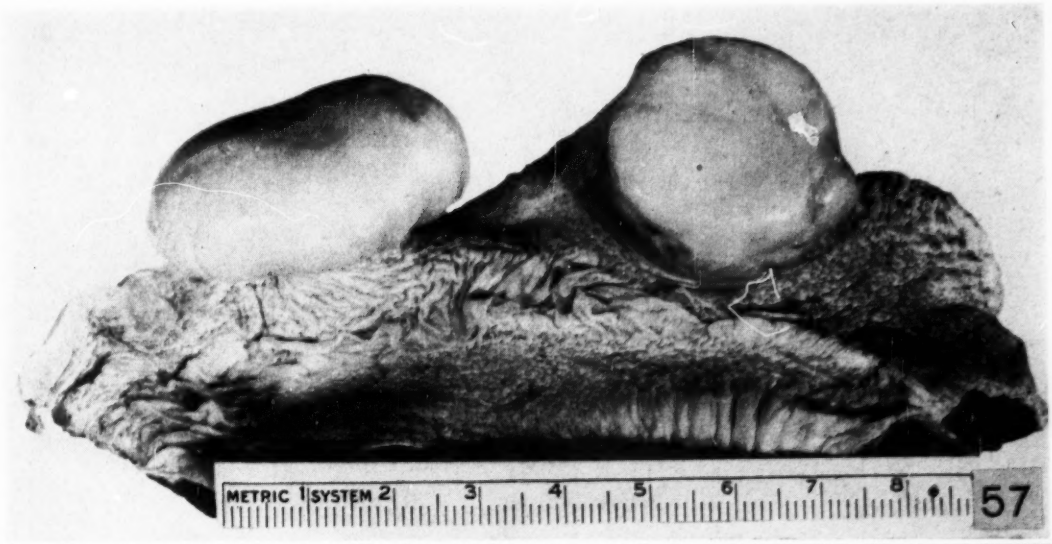
DESCRIPTION OF FIGURES 57 TO 59

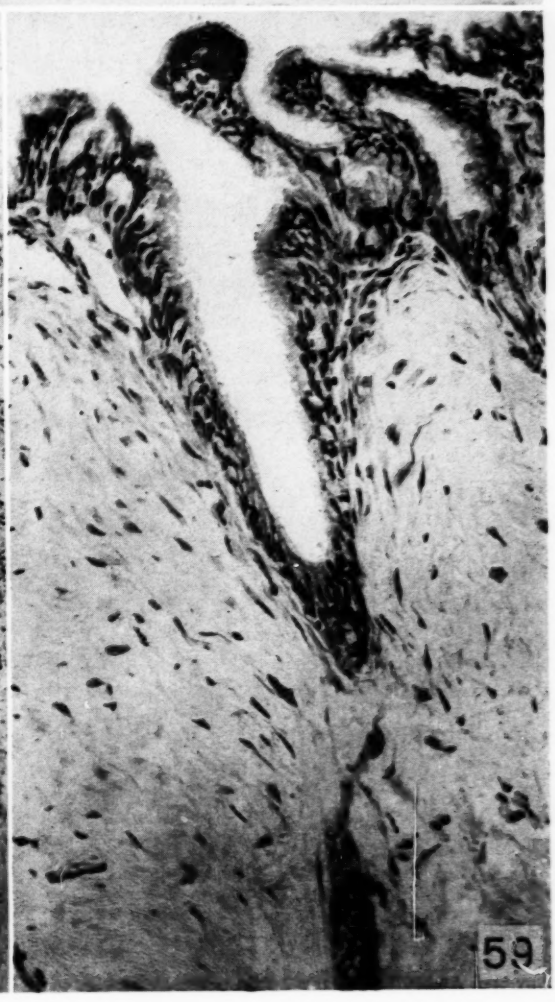
Fibromas of the lung in the green turtle, *Chelonia mydas*.

FIG. 57.—Two fibromas of the lung. After incision, the elastic pulmonary parenchyma retracted away from the tumors, leaving their surfaces smooth and glistening.

FIG. 58.—The fibroma occupies the left half of the photograph. There is no evidence of infiltration of the adjacent structures, but the alveoli of the lung are compressed. Mag. × 30.

FIG. 59.—The periphery of the tumor shown in the preceding figure. The surface is covered by normal ciliated respiratory epithelium. The underlying connective tissue comprises the bulk of the tumor; it is dense, partly hyalinized, and contains relatively few nuclei. Mag. × 150.





FIGS. 57-59

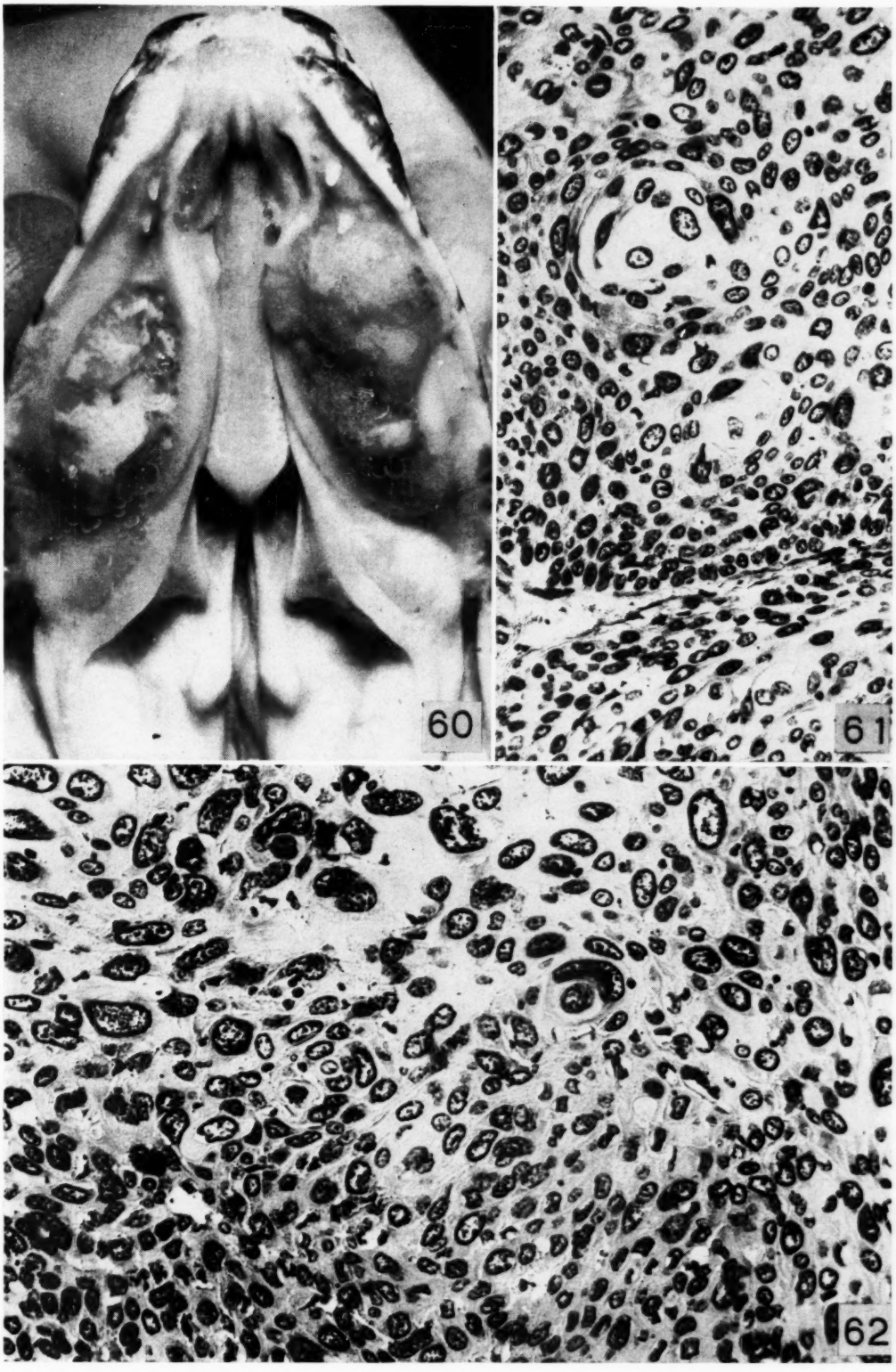
DESCRIPTION OF FIGURES 60 TO 62

Oral squamous cell carcinoma of a lizard, *Tupinambis nigropunctatus*.

FIG. 60.—Roof of the mouth in the living animal showing bilateral tumors. These arose in the epithelium of the gums and have completely destroyed several teeth. The white plaques are areas of necrosis.

FIG. 61.—In this section the neoplastic epithelial cells are in some areas arranged concentrically to form small nests or "pearls." Mag. × 200.

FIG. 62.—The nuclei of the tumor cells are remarkably large and pleomorphic; an occasional mitotic figure is present. Intercellular bridges can be seen. Mag. × 200.



FIGS. 60-62

DESCRIPTION OF FIGURES 63 TO 66

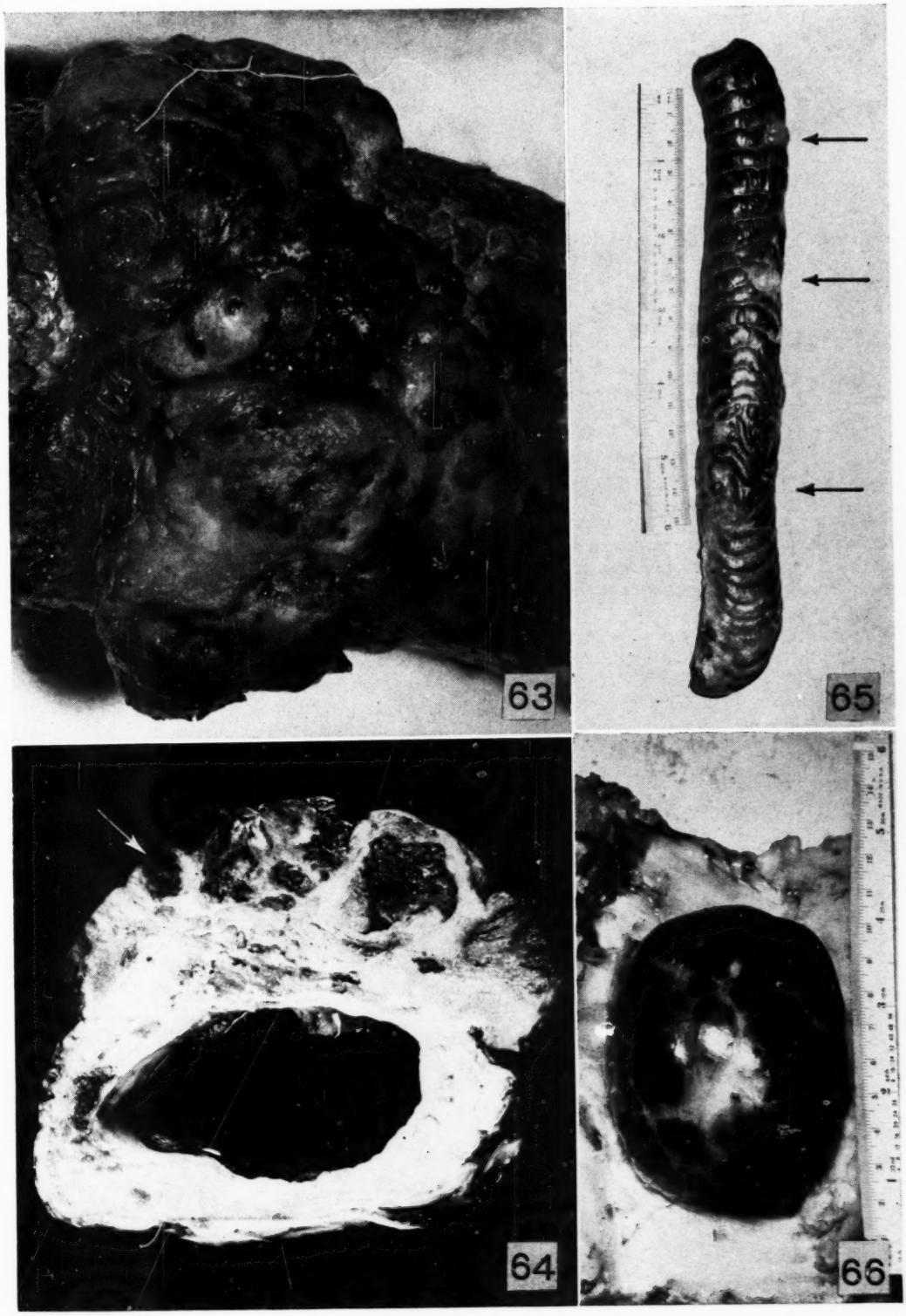
Malignant melanoma in a python, *Python reticulatus*.

FIG. 63.—Gross appearance of the primary tumor on the lateral aspect of the body of the snake. Note the absence of scales over the coarsely lobulated surface of the neoplasm. (Two-thirds natural size).

FIG. 64.—Cross section through the snake's body at the level of the tumor. The latter occupies about a third of the circumference of the body; it is coarsely lobulated, and contains several large areas of necrosis. Most of the tumor is non-pigmented; however, several densely pigmented areas are present (arrow). (One-half natural size.)

FIG. 65.—Kidney bearing three non-pigmented metastatic tumor nodules (arrows). (One-half natural size.)

FIG. 66.—A deeply pigmented metastatic tumor on the parietal surface of the coelomic cavity, 20 cm. caudal to the heart. (Natural size.)



FIGS. 63-66

DESCRIPTION OF FIGURES 67 TO 70

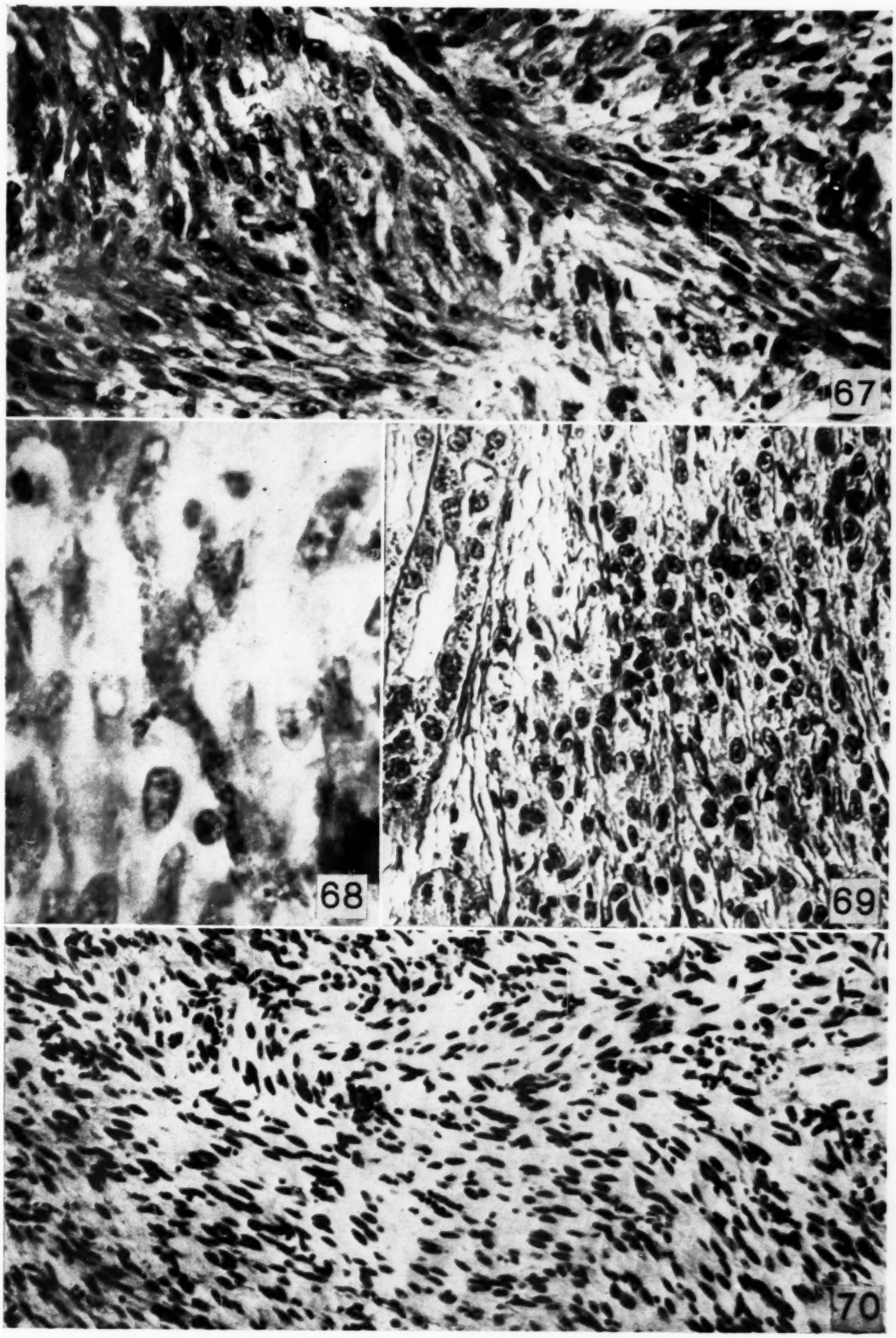
Histologic appearance of malignant melanoma in a python, *Python reticulatus*.

FIG. 67.—Section from the primary neoplasm showing the interlacing bundles of spindle shaped neoplastic cells. In this area, only an occasional cell bears pigment granules. Mag. × 350.

FIG. 68.—High power magnification of a neoplastic cell containing pigment. In the center of the photograph is an X-shaped cell with a large oval nucleus. Throughout the cytoplasm there is a uniform scattering of pigment granules. Mag. × 1300.

FIG. 69.—Metastatic tumor in the kidney. Along the left margin of the photograph is a normal renal tubule to the right of which is a sheet of neoplastic cells. The latter differ from those in the primary lesion in that they are less spindle shaped; the nuclei tend to be spherical. Mag. × 230.

FIG. 70.—A region from the primary tumor in which the cell pattern has a rather pronounced neurogenic appearance. There is a suggestion of palisading of the nuclei. Mag. × 200.



FIGS. 67-70

NOTICE

The Index to Literature of Experimental Cancer Research, 1900 to 1935, prepared by the Donner Foundation, is now available. It is a bound volume of over 1000 pages. The Donner Foundation has generously distributed copies to the principal medical libraries of the United States and Canada, and to some European libraries. Individuals wishing a copy for their personal use may obtain one by addressing the Donner Foundation, 2136 Land Title Building, Philadelphia 10, Pa. The price is $10, which is less than the cost of publication.

ERRATUM

In the paper by Bernhard, Rosenbloom and Eichen, entitled "A Study of Inorganic Phosphorus Release Accompanying Glycolysis of Blood in Cancer," Vol. 8, No. 6, p. 295, in the last line of the first paragraph, "252 mgm. per cent" should read "25 mgm. per cent."

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